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### INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT

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` ´	Priority Data: 98204291.3 16 December1998 (16  Parent Application or Grant UNIVERSITY OF LIEGE [/]; (). MELICA (). SEGHERSGENTEC N.V. [/]; (). ANDE (). GEORGES, Michel [/]; (). SPINCEMAI (). NEZER, Carine, Danielle, Andrée [/]; ()	A HB [ ERSSO LLE, (	[/]; DN, Leif [/]; Geen [/]:		
	Leif [/]; (). GEORGES, Michel [/]; (). SPIN [/]; (). NEZER, Carine, Danielle, Andrée [/] (). OTTEVANGERS, S., U.; ().	<b>ICEM</b>			

- (54) Title: SELECTING ANIMALS FOR PARENTALLY IMPRINTED TRAITS
- (54) Titre: SELECTION D'ANIMAUX EN FONCTION DE TRAITS COMMUNIQUES PAR LEURS PARENTS

### (57) Abstract

The invention relates to methods to select breeding animals or animals destined for slaughter for having desired genotypic or potential phenotypic properties, in particular related to muscle mass and/or fat deposition. The invention provides a method for selecting a pig for having desired genotypic or potential phenotypic properties comprising testing a sample from said pig for the presence of a quantitative trait locus (QTL) located at a Sus scrofa chromosome 2 mapping at position 2p1.7.

### (57) Abrégé

L'invention concerne des procédés de sélection d'animaux reproducteurs ou destinés à l'abattoir sur la base des propriétés génotypiques désirées ou des propriétés phénotypiques potentielles qui sont notamment liées à la masse musculaire et/ou aux dépôts de lard. L'invention se rapporte à un procédé pour sélectionner un porc possédant des propriétés génotypiques désirées ou des propriétés phénotypiques potentielles, ledit procédé consistant à tester un échantillon provenant dudit porc pour vérifier la présence d'un locus quantitatif (QTL) présent dans la cartographie de chromosome 2 de Sus scrofa en position 2p1.7.

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# INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(71) Applicants (for all designated States except US): UNIVER-SITY OF LIEGE [BE/BE]; 20 Bd de Colonster, B-4000 Liege (BE). MELICA HB [SE/SE]; Andersson, Leif, Bergagatan 30, S-752 39 Uppsala (SE). SEGHERSGENTEC N.V. [BE/BE]; Kapelbaan 15, B-9255 Buggenhout (BE).

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(75) Inventors, and (75) Inventors/Applicants (for US only): ANDERSSON, Leif [SE/SE]; Bergagatan 30, S-752 39 Uppsala (SE). GEORGES, Michel [BE/BE]; Rue Vieux Tige 24, B-3161 Villers-aux-Tours (BE). SPINCEMAILLE, Geert [BE/BE]; Sint Denijsstraat 26, B-8550 Zwevegem (BE).

(74) Agent: OTTEVANGERS, S., U.; Vereenigde, Nieuwe Parklaan 97, NL-2587 BN The Hague (NL).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, GW, ML, MR, NE, SN, TD, TG).

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(54) Title: SELECTING ANIMALS FOR PARENTALLY IMPRINTED TRAITS

The invention relates to methods to select breeding animals or animals destined for slaughter for having desired genotypic or potential phenotypic properties, in particular related to muscle mass and/or fat deposition. The invention provides a method for selecting a pig for having desired genotypic or potential phenotypic properties comprising testing a sample from said pig for the presence of a quantitative trait locus (QTL) located at a Sus scrofa chromosome 2 mapping at position 2p1.7.

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## Description

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Title: Selecting animals for parentally imprinted traits.

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been relied on so far.

The invention relates to methods to select breeding animals or animals destined for slaughter for having desired genotypic or potential phenotypic properties, in particular related to muscle mass and/or fat deposition. Breeding schemes for domestic animals have so far focused on farm performance traits and carcass quality. This has resulted in substantial improvements in traits like reproductive success, milk production, lean/fat ratio, prolificacy, growth rate and feed efficiency. Relatively simple performance test data have been the basis for these improvements, and selected traits were assumed to be influenced by a large number of genes, each of small effect (the infinitesimal gene model). There are now some important changes occurring in this area. First, the breeding goal of some breeding organisations has begun to include meat quality attributes in addition to the "traditional" production traits. Secondly, evidence is accumulating that current and new breeding goal traits may involve relatively large effects (known as major genes), as opposed to the infinitesimal model that has

Modern DNA-technologies provide the opportunity to exploit these major genes, and this approach is a very promising route for the improvement of meat quality, especially since direct meat quality assessment is not viable for potential breeding animals. Also for other traits such as lean/fat ratio, growth rate and feed efficiency, modern DNA technology can be very effective. Also these traits are not always easy to measure in the living animal.

The evidence for several of the major genes originally obtained using segregation analysis, i.e. without any DNA marker information. Afterwards molecular studies were performed to detect the location of these

genes on the genetic map. In practice, and except for alleles of very large effect, DNA studies are required to dissect the genetic nature of most traits of economic importance. DNA markers can be used to localise genes or 5 alleles responsible for qualitative traits like coat colour, and they can also be used to detect genes or alleles with substantial effects on quantitative traits like growth rate, IMF etc. In this case the approach is referred to as QTL (quantitative trait locus) mapping, wherein a QTL comprises at least a part of the nucleic acid genome of an animal where genetic information capable of influencing said quantitative trait (in said animal or in its offspring) is located. Information at DNA level can not only help to fix a specific major gene in a population, but also assist in the selection of a quantitative trait which is already selected for. Molecular information in addition to phenotypic data can

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Improving meat quality or carcass quality is not just about changing levels of traits like tenderness or marbling, but it is also about increasing uniformity. The existence of major genes provides excellent opportunities for improving meat quality because it allows large steps to be made in the desired direction. Secondly, it will help to reduce variation, since we can fix relevant genes in our products. Another aspect is that selecting for major genes allows differentiation for specific markets. Studies are underway in several species, particularly, pigs, sheep, deer and beef cattle.

increase the accuracy of selection and therefore the

selection response.

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In particular, intense selection for meat production has resulted in animals with extreme muscularity and leanness in several livestock species. In recent years it has become feasible to map and clone several of the genes causing these phenotypes, paving the way towards more efficient marker assisted selection, targeted drug development (performance enhancing products) and transgenesis. Mutations in the ryanodine receptor (Fuji

et al, 1991; MacLennan and Phillips, 1993) and myostatin (Grobet et al, 1997; Kambadur et al, 1997; McPherron and Lee, 1997) have been shown to cause muscular hypertrophies in pigs and cattle respectively, while genes with major effects on muscularity and/or fat deposition have for instance been mapped to pig chromosome 4 (Anderssor et al, 1994) and sheep chromosome 18 (Cocket et al, 1996).

However, although there have been successes in

identifying QTLs, the information is currently of limited use within commercial breeding programmes. Many workers in this field conclude that it is necessary to identify the particular genes underlying the QTL. This is a substantial task, as the QTL region is usually relatively large and may contain many genes. Identification of the relevant genes from the many that may be involved thus remains a significant hurdle in farm animals.

The invention provides a method for selecting a

20 domestic animal for having desired genotypic or potential
phenotypic properties comprising testing said animal for
the presence of a parentally imprinted qualitative or
quantitative trait locus (QTL). Herein, a domestic animal
is defined as an animal being selected or having been

25 derived from an animal having been selected for having
desired genotypic or potential phenotypic properties.

Domestic animals provide a rich resource of genetic and phenotypic variation, traditionally domestication involves selecting an animal or its offspring for having desired genotypic or potential phenotypic properties. This selection process has in the past century been facilitated by growing understanding and utilisation of the laws of Mendelian inheritance. One of the major problems in breeding programs of domestic animals is the negative genetic correlation between reproductive capacity and production traits. This is for example the case in cattle (a high milk production generally results)

in slim cows and bulls) poultry, broiler lines have a low level of egg production and layers have generally very low muscle growth), pigs (very prolific sows are in general fat and have comparatively less meat) or sheep 10 (high prolific breeds have low carcass quality and vice versa). The invention now provides that knowledge of the parental imprinting character of various traits allows to select for example sire lines homozygous for a paternally 15 imprinted QTL for example linked with muscle production or growth; the selection for such traits can thus be less stringent in dam lines in favour of the reproductive . quality. The phenomenon of genetic or parental imprinting 20 has never been utilised in selecting domestic animals, it was never considered feasible to employ this elusive genetic characteristic in practical breeding programmes. The invention provides a breeding programme, wherein 25 knowledge of the parental imprinting character of a desired trait, as demonstrated herein, results in a breeding programme, for example in a BLUP programme, with a modified animal model. This increases the accuracy of 20 30 the breeding value estimation and speeds up selection compared to conventional breeding programmes. Until now, the effect of a parentally imprinted trait in the estimation of a conventional BLUP programme was 35 neglected; using and understanding the parental character of the desired trait, as provided by the invention, allows selecting on parental imprinting, even without DNA testing. For example, selecting genes characterised by 40 paternal imprinting is provided to help increase uniformity; a (terminal) parent homozygous for the "good 30 or wanted" alleles will pass them to all offspring, regardless of the other parent's alleles, and the offspring will all express the desired parent's alleles. 45 This results in more uniform offspring. Alleles that are interesting or favourable from the maternal side or often the ones that have opposite effects to alleles from the paternal side. For example, in meat animals such as pigs 50 alleles linked with meat quality traits such as inta-

muscular fat or muscle mass could be fixed in the dam lines while alleles linked with reduced back fat could be fixed in the sire lines. Other desirable combinations are for example fertility and/or milk yield in the female line with growth rates and/or muscle mass in the male lines.

In a preferred embodiment, the invention provides a method for selecting a domestic animal for having desired genotypic or potential phenotypic properties comprising testing a nucleic acid sample from said animal for the presence of a parentally imprinted quantitative trait locus (QTL). A nucleic acid sample can in general be obtained from various parts of the animal's body by methods known in the art. Traditional samples for the purpose of nucleic acid testing are blood samples or skin or mucosal surface samples, but samples from other tissues can be used as well, in particular sperm samples, oocyte or embryo samples can be used. In such a sample, the presence and/or sequence of a specific nucleic acid, be it DNA or RNA, can be determined with methods known in the art, such as hybridisation or nucleic acid amplification or sequencing techniques known in the art. The invention provides testing such a sample for the presence of nucleic acid wherein a QTL or allele associated therewith is associated with the phenomenon of parental imprinting, for example where it is determined whether a paternal or maternal allele of said QTL is capable of being predominantly expressed in said animal.

The purpose of breeding programs in livestock is to enhance the performances of animals by improving their genetic composition. In essence this improvement accrues by increasing the frequency of the most favourable alleles for the genes influencing the performance characteristics of interest. These genes are referred to as QTL. Until the beginning of the nineties, genetic improvement was achieved via the use of biometrical methods, but without molecular knowledge of the underlying QTL.

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Since the beginning of the nineties and due to recent developments in genomics, it is conceivable to identify the QTL underlying a trait of interest. The invention now provides identifying and using parentally imprinted QTLs which are useful for selecting animals by mapping quantitative trait loci. Again, the phenomenon of genetic or paternal imprinting has never been utilised in selecting domestic animals, it was never considered feasible to employ this elusive genetic characteristic in practical breeding programmes. For example Kovacs and

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practical breeding programmes. For example Kovacs and Kloting (Biochem. Mol. Biol. Int. 44:399-405, 1998), where parental imprinting is not mentioned, and not suggested, found linkage of a trait in female rats, but not in males, suggesting a possible sex specificity associated with a chromosomal region, which of course excludes parental imprinting, a phenomenon wherein the imprinted trait of one parent is preferably but gender-aspecifically expressed in his or her offspring.

The invention provides the initial localisation of a parentally imprinted QTL on the genome by linkage analysis with genetic markers, and the actual identification of the parentally imprinted gene(s) and causal mutations therein. Molecular knowledge of such a parentally imprinted QTL allows for more efficient breeding designs herewith provided. Applications of molecular knowledge of parentally imprinted QTLs in breeding programs include: marker assisted segregation analysis to identify the segregation of functionally distinct parentally imprinted QTL alleles in the populations of interest, marker assisted selection (MAS) performed within lines to enhance genetic response by increasing selection accuracy, selection intensity or by reducing the generation interval using the understanding of the phenomonon of parental imprinting, marker assisted introgression (MAI) to efficiently transfer favourable parentally imprinted QTL alleles from a donor to a recipient population, genetic engineering of the identified parentally QTL and genetic modification of the . breeding stock using transgenic technology, development

of performance enhancing products using targeted drug development exploiting molecular knowledge of said QTL.

The inventors undertook two independent experiments to determine the practical use of parental imprinting of a OTL.

In a first experiment, performed in a previously described Piétrain x Large White intercross, the likelihood of the data were computed under a model of paternal (paternal allele only expressed) and maternal imprinting (maternal allele only expressed) and compared with the likelihood of the data under a model of a conventional "Mendelian" QTL. The results strikingly demonstrated that the QTL was indeed paternally expressed, the QTL allele (Piétrain or Large White) inherited from the F1 sow having no effect whatsoever on the carcass quality and quantity of the F2 offspring. It was seen that very significant lodscores were obtained when testing for the presence of a paternally expressed QTL, while there was no evidence at all for the segregation of a QTL when studying the chromosomes transmitted by the sows. The same tendency was observed for all traits showing that the same imprinted gene is responsible for the effects observed on the different traits. Table 1 reports the maximum likelihood (ML) phenotypic means for the  $F_2$  offspring sorted by inherited paternal QTL allele.

In a second experiment performed in the Wild Boar X Large White intercross, QTL analyses of body composition, fatness, meat quality, and growth traits was carried out with the chromosome 2 map using a statistical model testing for the presence of an imprinting effect. Clear evidence for a paternally expressed QTL located at the very distal tip of 2p was obtained (Fig. 2; Tablel). The clear paternal expression of a QTL is illustrated by the least squares means which fall into two classes following the population origin of the paternally inherited allele (Table 1). For a given paternally imprinted QTL, implementation of marker assisted segregation analysis, selection (MAS) and introgression (MAI), can be performed

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using genetic markers that are linked to the QTL, genetic

markers that are in linkage disequilibrium with the QTL, or using the actual causal mutations within the QTL.

Understanding the parent-of-origin effect characterising a QTL allows for its optimal use in breeding programs. Indeed, marker assisted segregation analysis under a model of parental imprinting will yield better estimates of QTL allele effects. Moreover it allows for the application of specific breeding schemes to optimally exploit a QTL. In one embodiment of the invention, the most favourable QTL alleles would be fixed in breeding animal lines and for example used to generate commercial, crossbred males by marker assisted selection (MAS, within lines) and marker assisted introgression (MAI, between lines). In another embodiment, the worst QTL alleles would be fixed in the animal lines used to generate commercial crossbred females by MAS (within lines) and MAI (between lines).

In a preferred embodiment of the invention, said animal is a pig. Note for example that the invention provides the insight that today half of the offspring from commercially popular Piétrain, Large White crossbred boars inherit an unfavourable Large White muscle mass QTL as provided by the invention causing considerable loss, and the invention now for example provides the possibility to select the better half of the population in that respect. However, it is also possible to select commercial sow lines enriched with the in the boars unfavourable alleles, allowing to equip the sows with other alleles more desirable for for example reproductive purposes.

In a preferred embcdiment of a method provided by the invention, said QTL is located at a position corresponding to a QTL located at chromosome 2 in the pig. For example, it is known form comparative mapping data between pig and human, including bidirectional chromosome painting, that SSC2p is homologous to HSAllpter-q13<sup>11,12</sup>. HSAllpter-q13 is known to harbour a

cluster of imprinted genes: IGF2, INS2, H19, MAH2, P57\*1P2, K,LQTL1, Tapal,/CD81, Orctl2, Impt1 and Ip1. The cluster of imprinted genes located in HSAllpter-ql3 is characterised by 8 maternally expressed genes H19, MASH2, P57KIP2, K,LQTL1, TAPA1/CD81, ORCTL2, IMPT1 and IP1, and 10 two paternally expressed genes: IGF2 and INS. However, Johanson et al (Genomics 25:682-690, 1995) and Reik et al (Trends in Genetics, 13:330-334, 1997) show that the whereabouts of these loci in various animals are not 15 clear. For example, the HSA11 and MMU7 loci do not correspond among each other, the MMU7 and the SSC2 loci do not correspond, whereas the HSAll and SSC2 loci seem to correspond, and no guidance is given where one or more 20 of for example the above identified parentally expressed individual genes are localised on the three species' chromosomes. Other domestic animals, such as cattle, sheep, 25

poultry and fish, having similar regions in their genome harbouring such a cluster of imprinted genes or QTLs, the invention herewith provides use of these orthologous regions of other domestic animals in applying the phenomenon of parental imprinting in breeding programmes. In pigs, said cluster is mapped at around position 2p1.7 of chromosome 2, however, a method as provided by the invention employing (fragments of) said maternally or paternally expressed orthologous or homologous genes or QTLs are advantageously used in other animals as well for breeding and selecting purposes. For example, a method is provided wherein said QTL is related to the potential muscle mass and/or fat deposition, preferably with limited effects on other traits such as meat quality and daily gain of said animal or wherein said QTL comprises at least a part of an insulin-like growth factor-2 (IGF2) allele. Reik et al (Trends in Genetics, 13:330-334, 1997) explain that this gene in humans is related to Beckwith-Wiedemann syndrome, an apparently parentally imprinted disease syndrome most commonly seen with human foetuses, where the gene has an important role in prenatal

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development. No relationship is shown or suggested with postnatal development relating to muscle development or fatness in (domestic) animals.

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In a preferred embodiment, the invention provides a method for selecting a pig for having desired genotypic or potential phenotypic properties comprising testing a sample from said pig for the presence of a quantitative trait locus (QTL) located at a Sus scrofa chromosome 2 mapping at position 2p1.7. In particular, the invention relates to the use of genetic markers for the telomeric end of pig chromosome 2p in marker selection (MAS) of a parentally imprinted Quantitative Trait Locus (QTL) affecting carcass yield and quality in pigs. Furthermore, the invention relates to the use of genetic markers associated with the IGF2 locus in MAS in pigs, such as polymorphisms and microsatelites and other characterising nucleic acid sequences shown herein, such as shown in figures 4 to 10. In a preferred embodiment, the invention provides a QTL located at the distal tip of Sus scrofa chromosomes 2 with effects on varies measurements of carcass quality and quantity, particularly muscle mass and fat deposition.

In a first experiment, a QTL mapping analysis was performed in a Wild Boar X Large White intercross counting 200 F, individuals. The F, animals were sacrificed at a live eight of at least 80 kg or at a maximum age of 190 days. Phenotypic data on birth weight, growth, fat deposition, body composition, weight of internal organs, and meat quality were collected; a detailed description of the phenotypic traits are provided by Andersson et al and Andersson-Eklund et al.

A QTL (without any significant effect on back-fat thickness) at an unspecified locus on the proximal end of chromosome 2 with moderate effect on muscle mass, and located about 30cM away from the parentally imprinted QTL reported here, was previously reported by the inventors; whereas the QTL as now provided has a very large effect, explaining at least 20-30% of variance, making the QTL of

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the present invention commercially very attractive, which is even more so because the present QTL is parentally imprinted. The marker map of chromosome 2p was improved as part of this invention by adding microsatellite markers in order to cover the entire chromosome arm. The following microsatellite markers were used: Swc9, Sw2443, Sw2623, and Swr2516, all from the distal end of 2p1. QTL analyses of body composition, fatness, meat quality, and growth traits were carried out with the new chromosome 2 map. Clear evidence for a QTL located at the very distal tip of 2p was obtained (Fig. 1; Table 1). The QTL had very large effects on lean meat content in ham and explained an astonishing 30% of the residual phenotypic variance in the F2 population. Large effects on the area of the longissumus dorsi muscle, on the weight of the heart, and on back-fat thickness (subcutaneous fat) were also noted. A moderate effect on one meat quality trait, reflectance value, was indicated. The QTL had no significant effect on abdominal fat, birth weight, growth, weight of liver, kidney, or spleen (data not shown). The Large White allele at this QTL was associated with larger muscle mass and reduced back-fat thickness consistent with the difference between this breed and the Wild Boar population. In a second experiment, QTL mapping was performed in 25

In a second experiment, QTL mapping was performed in a Piétrain X Large White intercross comprising 1125 F<sub>2</sub> offspring. The Large White and Piétrain parental breeds differ for a number of economically important phenotypes. Piétrains are famous for their exceptional muscularity and leanness <sup>10</sup>(Figure 2, while Large Whites show superior growth performance. Twenty-one distinct phenotypes measuring growth performance (5), muscularity (6), fat deposition (6), and meat quality (4), were recorded on all F<sub>2</sub> offspring. In order to map QTL underlying the genetic differences between these breeds, the inventors undertook a whole genome scan using microsatellite markers on an initial sample of 677 F<sub>2</sub> individuals. The following microsatellite marker map was used to analyse

chromosome 2;:SW2443, SWC9 and SW2623, SWR2516-(0,20)-5 SWR783-(0,29)-SW240-(0,20)-SW776-(0,08)-S0010-(0,04)-SW1695-(0,36)-SWR308. Analysis of pig chromosome 2 using a Maximum Likelihood multipoint algorithm, revealed 5 highly significant lodscores (up to 20) for three of the 10 six phenotypes measuring muscularity (% lean cuts, % ham, % loin) and three of the six phenotypes measuring fat deposition (back-fat thickness (BFT), % backfat, % fat cuts) at the distal end of the short arm of chromosome 2 15 (Figure 1). Positive lodscores were obtained in the 10 corresponding chromosome region for the remaining six muscularity and fatness phenotypes, however, not reaching the experiment-wise significance threshold ) ( $\alpha$ =5%. There 20 was no evidence for an effect of the corresponding QTL on 15. growth performance (including birth weight) or recorded meat quality measurements (data not shown). To confirm this finding, the remaining sample of 355 F, offspring was 25 genotyped for the four most distal 2p markers and QTL analysis performed for the traits yielding the highest lodscores in the first analysis. Lodscores ranged from 2.1 to 7.7, clearly confirming the presence of a major 30 QTL in this region. Table 2 reports the corresponding ML estimates for the three genotypic means as well as the residual variance. Evidence based on marker assisted segregation analysis points towards residual segregation 25 35 at this locus within the Piétrain population. These experiments therefore clearly indicated the existence of a QTL with major effect on carcass

These experiments therefore clearly indicated the existence of a QTL with major effect on carcass quality and quantity on the telomeric end of pig chromosome arm 2p; the likely existence of an allelic series at this QTL with at least three alleles: Wild-Boar < Large White < Piétrain, and possibly more given the observed segregation within the Piétrain breed.

The effects of the identified QTL on muscle mass and fat deposition are truly major, being of the same magnitude of those reported for the CRC locus though apparently without the associated deleterious effects on meat quality. We estimate that both loci jointly explain

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close to 50% of the Piétrain versus Large White breed difference for muscularity and leanness. The QTL had very large effects on lean meat content in ham and explained an astonishing 30% of the residual phenotypic variance in the F2 population. Large effects on the area of the

longissumus dorsi muscle, on the weight of the heart, and on back-fat thickness (subcutaneous fat) were also noted. A moderate effect on one meat quality trait, reflectance value, was indicated. The QTL had no significant effect

on abdominal fat, birth weight, growth, weight of liver, kidney, or spleen (data not shown). The Large White allele at this QTL, when compared to the Wild Boar allele, was associated with larger muscle mass and reduced back-fat thickness consistent with the difference

between this breed and the Wild Boar population. The strong imprinting effect observed for all affected traits shows that a single causative locus is involved. The pleiotropic effects on skeletal muscle mass and the size of the heart appear adaptive from a physiological point of view as a larger muscle mass requires a larger cardiac

output.

In a further embodiment, the invention provides a method for selecting a pig for having desired genotypic or potential phenotypic properties comprising testing a sample from said pig for the presence of a quantitative trait locus (QTL) located at a Sus scrofa chromosome 2 mapping at position 2pl.7., wherein said QTL comprises at least a part of a Sus scrofa insulin-like growth factor-2 (IGF2) allele or a genonic area closely related thereto, such as polymorphisms and microsatelites and other characterising nucleic acid sequences shown herein, such as shown in figures 4 to 10. The important role of IGF2 for prenatal development is well-documented from knock-out mice as well as from its causative role in the human Beckwith-Wiedemann syndrome. This invention demonstrates

Beckwith-Wiedemann syndrome. This invention demonstrates an important role for the *IGF2*-region also for postnatal development.

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To show the role of Igf2 the inventors performed the following three experiments:

A genomic IGF2 clone was isolated by screening a porcine BAC library. FISH analysis with this BAC clone gave a strong consistent signal on the terminal part of chromosome 2p.

A polymorphic microsatellite is located in the 3'UTR of IGF2 in mice (GenBank U71085), humans (GenBank S62623), and horse (GenBank AF020598). The possible presence of a corresponding porcine microsatellite was investigated by direct sequencing of the IFG2 3'UTR using the BAC clone. A complex microsatellite was identified about 800bp downstream of the stop codon; a sequence comparison revealed that this microsatellite was identical to a previously described anonymous microsatellite, Swc96. This marker was used in the initial QTL mapping experiments and its location on the genetic map correspond with the most likely position of the QTL both in the Piétrain X Large White and in the Large White x Wild Boar pedigree.

Analysis of skeletal muscle and liver cDNA from 10-week old foetuses heterozygous for a nt241 (G-A) transversion in the second exon of the porcine IGFII gene and SWC9, shows that the IGFII gene is imprinted in these tissues in the pig as well and only expressed from the paternal allele.

Based on a published porcine adult liver cDNA sequence 16, the inventors designed primer pairs allowing to amplify the entire IgfII coding sequence with 222 bp of leader and 280 bp of trailor sequence from adult skeletal muscle cDNA. Piétrain and Large White RT-PCR products were sequenced indication that the coding sequences are identical in both breeds and with the published sequence. However, a  $G \boxtimes A$  transition was found in the leader sequence corresponding to exon 2 in man. Following conventional nomenclature, this polymorphism will be referred to as nt241(G-A). We developed a screening test for this single nucleotide polymorphism

9(SNP) based on the ligation amplification reaction (LAR), allowing us to genotype our pedigree material. Based on these data, IgfII was shown to colocalize with the SWC9 microsatellite marker (θ=0%), therefore virtually coinciding with the most likely position of the QTL, and well within the 95% support interval for the QTL. Subsequent sequence analysis demonstrated that the microsatellite marker SWC9 is actually located within the 3'UTR of the IgfII gene.

As previously mentioned, the knowledge of this QTL provides a method for the selection of animals such as pigs with improved carcass merit. Different embodiments of the invention are envisaged, including: marker assisted segregation analysis to identify the segregation of functionally distinct QTL alleles in the populations of interest; marker assisted selection (MAS) performed within lines to enhance genetic response by increasing selection accuracy, selection intensity or by reducing the generation interval; marker assisted introgression (MAI) to efficiently transfer favourable QTL alleles from a donor to a recipient population; thereby enhancing genetic response in the recipient population. Implementation of embodiments marker assisted segregation analysis, selection (MAS) and introgression (MAI), can be performed using genetic markers that are linked to the QTL; genetic markers that are in linkage disequilibrium with the QTL, the actual causal mutations within the OTL.

In a further embodiment, the invention provides a method for selecting a pig for having desired genotypic or potential phenotypic properties comprising testing a sample from said pig for the presence of a quantitative trait locus (QTL) located at a Sus scrofa chromosome 2 mapping at position 2pl.7., wherein said QTL is paternally expressed, i.e. is expressed from the paternal allele. In man and mouse, Igf2 is known to be imprinted and to be expressed exclusively from the paternal allele in several tissues. Analysis of skeletal muscle cDNA from

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pigs heterozygous for the SNP and/or SWC9, shows that the same imprinting holds in the pig as well. Understanding the parent-of-origin effect characterising the QTL as provided by the invention now allows for its optimal use in breeding programs. Indeed, today half of the offspring from commercially popular Piétrain x Large White crossbred boars inherit the unfavourable Large White allele causing considerable loss. Using a method as provide by the invention avoids this problem.

The invention furthermore provides an isolated and/or recombinant nucleic acid or functional fragment derived thereof comprising a parentally imprinted quantitative trait locus (QTL) or fragment thereof capable of being predominantly expressed by one parental allele. Having such a nucleic acid as provided by the invention available allows constructing transgenic animals wherein favourable genes are capable of being exclusively or predominantly expressed by one parental allele, thereby equipping the offspring of said animal homozygous for a desired trait with desired properties related to that parental allele that is expressed.

In a preferred embodiment, the invention provides an isolated and/or recombinant nucleic acid or fragment derived thereof comprising a synthetic parentally imprinted quantitative trait locus (QTL) or functional fragment thereof derived from at least one chromosome. Synthetic herein describes a parentally expressed QTL wherein various elements are combined that originate from distinct locations from the genome of one or more animals. The invention provides recombinant nucleic acid wherein sequences related to parental imprinting of one QTL are combined with sequences relating to genes or favourable alleles of a second QTL. Such a gene construct is favourably used to obtain transgenic animals wherein the second QTL has been equipped with paternal imprinting, as opposed to the inheritance pattern in the native animal from which the second QTL is derived. Such a second QTL can for example be derived from the same

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chromosome where the parental imprinting region is located, but can also be derived from a different chromosome from the same or even a different species. In the pig, such a second QTL can for example be related to an oestrogen receptor (ESR)-gene (Rothschild et al, PNAS, 93, 201-201, 1996) or a FAT-QTL (Andersson, Science, 263, 1771-1774, 1994) for example derived from an other pig chromosome, such as chromosome 4. A second or further QTL can also be derived from another (domestic) animal or a human.

The invention furthermore provides an isolated and/or recombinant nucleic acid or functional fragment derived thereof at least partly corresponding to a QTL of a pig located at a Sus scrofa chromosome 2 mapping at position 2p1.7 wherein said QTL is related to the potential muscle mass and/or fat deposition of said pig and/or wherein said QTL comprises at least a part of a Sus scrofa insulin-like growth factor-2 (IGF2) allele, preferably at least spanning a region between INS and H19, or preferably derived from a domestic pig, such as a Pietrain, Meishan, Duroc, Landrace or Large White, or from a Wild Boar. For example, a genomic IGF2 clone was isolated by screening a porcine BAC library. FISH analysis with this BAC clone gave a strong consistent signal on the terminal part of chromosome 2p. A polymorphic microsatellite is located in the 3'UTR of IGF2 in mice (GenBank U71085), humans (GenBank S62623), and horse (GenBank AF320598). The possible presence of a corresponding porcine microsatellite was investigated by direct sequencing of the IGF2 3'UTR using the BAC clone. A complex microsatellite was identified about 800 bp downstream of the stop codon; a sequence comparison revealed that this microsatellite is identical to a previously described anonymous microsatellite, Swc9. PCR primers were designed and the microsatellite ( $\mathit{IGF2ms}$ ) was found to be highly polymorphic with three different alleles among the two Wild Boar founders and another two

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among the eight Large White founders. IGF2ms was fully informative in the intercross as the breed of origin as well as the parent of origin could be determined with confidence for each allele in each  $F_2$  animal.

A linkage analysis using the intercross pedigree was carried out with IGF2ms and the microsatellites Sw2443, Sw2623, and Swr2516, all from the distal end of  $2p^7$ . IGF2 was firmly assigned to 2p by highly significant lod scores (e.g. Z=89.0,  $\theta=0.003$  against Swr2516). Multipoint analyses, including previously typed chromosome 2 markers, revealed the following order of loci (sexaverage map distances in Kosambi cM): Sw2443/Swr2516-0.3-IGF2-14.9-Sw2623-10.3-Sw256. No recombinant was observed between Sw2443 and Swr2516, and the suggested proximal location of IGF2 in relation to these loci is based on a single recombinant giving a lod score support of 0.8 for the reported order. The most distal marker in our previous QTL study, Sw256, is located about 25 cM from the distal end of the linkage group.

The invention furthermore provides use of a nucleic acid or functional fragment derived thereof according to the invention in a method according to the invention. In a preferred embodiment, use of a method according to invention is provided to select a breeding animal or animal destined for slaughter, or embryos or semen derived from these animals for having desired genotypic or potential phenotypic properties. In particular, the invention provides such use wherein said properties are related to muscle mass and/or fat deposition. The QTL as provided by the invention may be exploited or used to improve for example lean meat content or back-fat thickness by marker assisted selection within populations or by marker assisted introgression of favorable alleles from one population to another. Examples of marker assisted selection using the QTL as provided by the

invention are use of marker assisted segregation analysis

with linked markers or with markers in disequilibrium to identify functionally distinct QTL alleles. Furthermore, identification of a causative mutation in the QTL is now possible, again leading to identify functionally distinct 5 QTL alleles. Such functionally distinct QTL alleles 10 located at the distal tip of chromosome 2p with large effects on skeletal muscle mass, the size of the heart, and on back-fat thickness are also provided by the invention. The observation of a similar QTL effect in a 15 10 Large White x Wild Boar as well as in a Piétrain x Large White intercross provides proof of the existence of a . series of at least three distinct functional alleles. 20 Moreover, preliminary evidence based on marker assisted segregation analysis points towards residual segregation 15 at this locus within the Piétrain population (data not shown). The occurrence of an allelic series as provided 25 by the invention allows identifying causal polymorphisms which - based on the quantitative nature of the observed effect - are unlikely to be gross gene alterations but rather subtle regulatory mutations. The effects on muscle 20 30 mass of the three alleles rank in the same order as the breeds in which they are found i.e. Piétrain pigs are more muscular than Large White pigs that in turn have higher lean meat content than Wild Boars. The invention 35 furthermore provides use of the alleles as provided by the invention for within line selection or for marker assisted introgression using linked markers, markers in disequilibrium or alleles comprising causative mutations. 40 The invention furthermore provides an animal

The invention furthermore provides an animal selected by using a method according to the invention. For example, a pig characterised in being homozygous for an allele in a QTL located at a Sus scrofa chromosome 2 mapping at position 2pl.7 can now be selected and is thus provided by the invention. Since said QTL is related to the potential muscle mass and/or fat deposition of said pig and/or said QTL comprises at least a part of a Sus scrofa insulin-like growth factor-2 (IGF2) allele, it is

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possible to select promising pigs to be used for breeding or to be slaughtered. In particular an animal according to the invention which is a male is provided. Such a male, or its sperm or an embryo derived thereof can advantageously be used in breeding animals for creating breeding lines or for finally breeding animals destined for slaughter. In a preferred embodiment of such use as provided by the invention, a male, or its sperm, deliberately selected for being homozygous for an allele causing the extreme muscular hyperthrophy and leanness, is used to produce offspring heterozygous for such an allele. Due to said allele's paternal expression, said offspring will also show the favourable traits for example related to muscle mass, even if the parent female has a different genetic background. Moreover, it is now possible to positively select the female(s) for having different traits, for example related to fertility, without having a negative effect on the muscle mass trait that is inherited from the allele from the selected male. For example, earlier such males could occasionally be seen with Piétrain pigs but genetically it was not understood how to most profitably use these traits in breeding programmes.

Furthermore, the invention provides a transgenic animal, sperm and an embryo derived thereof, comprising a synthetic parentally imprinted QTL or functional fragment thereof as provided by the invention, i.e. it is provided by the invention to introduce a favourable recombinant allele; for example introduce the oestrogen receptor locus related to increased litter size of an animal homozygously in a parentally imprinted region of a grandparent animal (for example the father of a hybrid sow if the region was paternally imprinted and the grandparent was a boar); to introduce a favourable fatrelated allele or muscle mass-related recombinant allele in a paternally imprinted region, and so on. Recombinant alleles that are interesting or favourable from the maternal side or often the ones that have opposite effects to alleles from the paternal side. For example,

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described<sup>25</sup>. The final concentration of the probe in the hybridization mix was 10 ng/ $\mu$ l. Repetitive sequences were

suppressed with standard concentrations of porcine

in meat animals such as pigs recombinant alleles linked 5 with meat quality traits such as intra-muscular fat or muscle mass could be fixed in the dam lines while recombinant alleles linked with reduced back fat could be fixed in the sire lines. Other desirable combinations are 10 for example fertility and/or milk yield in the female line with growth rates and/or muscle mass in the male lines. The invention is further explained in the detailed 15 description without limiting the invention. 10 Detailed description. 20 Example 1: Wild Boar x Large White intercrosses 15 Methods 25 Isolation of an IGF2 BAC clone and fluorescent in situ hybridization (FISH). IGF2 primers (F:5'-20 GGCAAGTTCTTCCGCTAATGA-3' and R:5'-GCACCGCAGAATTACGACAA-3') for PCR amplification of a part of the last exon and 30 3'UTR were designed on the basis of a porcine IGF2 cDNA sequence (GenBank X56094). The primers were used to screen a porcine BAC library and the clone 253G10 was 35 isolated. Crude BAC DNA was prepared as described24. The BAC DNA was linearized with EcoRV and purified with QIAEXII (QIAGEN GmbH, Germany). The clone was labeled with biotin-14-dATP using the GIBCO-BRL Bionick labeling 40 system (BRL18246-015). Porcine metaphase chromosomes were obtained from pokeweed (Seromed) stimulated lymphocytes using standard techniques. The slides were aged for two days at room temperature and then kept at -20°C until 45 use. FISH analysis was carried out as previously

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genomic DNA. After post-hybridization washing, the biotinylated probe was detected with two layers of avidin-FITC (Vector A-2011). The chromosomes were counterstained with 0.3~mg/ml DAPI (4,6-Diamino-2-1)

phenylindole; Sigma D9542), which produced a G-banding like pattern. No posthybridization banding was needed, since chromosome 2 is easily recognized without banding. A total of 20 metaphase spreads were examined under an Olympus BX-60 fluorescence microscope connected to an

10 IMAC-CCD S30 video camera and equipped with an ISIS 1.65 (Metasystems) software.

Sequence, microsatellite, and linkage analysis.

About two µg of linearized and purified BAC DNA was used for direct sequencing with 20 pmoles of primers and BigDye Terminator chemistry (Perkin Elmer, USA). DNA sequencing was cone from the 3' end of the last exon towards the 3' end of the UTR until a microsatellite was

20 detected. A primer set (F:5'-GTTTCTCCTGTACCCACACGCATCCC-3' and R:5'-Fluorescein- CTACAAGCTGGGCTCAGGG-3') was designed for the amplification of the IGF2 microsatellite which is about 250 bp long and located approximately 800 bp downstream from the stop codon. The microsatellite was PCR amplified using fluorescently labeled primers and the

genotyping was carried out using an ABI377 sequencer and the GeneScan/Genotyper softwares (Perkin Elmer, USA). Two-point and multipoint linkage analysis were done with the Cri-Map software  $^{26}$ .

Animals and phenotypic data.

The intercross pedigree comprised two European Wild Boar males and eight Large White females, 4  $F_1$  males and 22  $F_2$ 

females, and 200  $F_2$  progeny<sup>1</sup>. The  $F_2$  animals were sacrificed at a live weight of at least 80 kg or at a

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maximum age of 190 days. Phenotypic data on birth weight, growth, fat deposition, body composition, weight of internal organs, and meat quality were collected; a detailed description of the phenotypic traits are provided by Andersson  $et\ al.^1$  and Andersson-Eklund et

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Statistical analysis.

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10 Interval mapping for the presence of QTL were carried out with a least squares method developed for the analysis of crosses between outbred lines<sup>27</sup>. The method is based on the assumption that the two divergent lines are fixed for alternative QTL alleles. There are four possible

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genotypes in the  $F_2$  generation as regards the grandparental origin of the alleles at each locus. This makes it possible to fit three effects: additive, dominance, and imprinting<sup>2</sup>. The latter is estimated as the difference between the two types of heterozygotes,

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the one receiving the Wild Boar allele through an  $F_1$  sire and the one receiving it from an  $F_1$  dam. An F-ratio was calculated using this model (with 3 d.f.) versus a reduced model without a QTL effect for each cM of chromosome 2. The most likely position of a QTL was

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obtained as the location giving the highest F-ratio.

Genome-wise significance thresholds were obtained empirically by a permutation test<sup>28</sup> as described<sup>2</sup>. The QTL model including an imprinting effect was compared with a model without imprinting (with 1 d.f.) to test

whether the imprinting effect was significant.

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The statistical models also included the fixed effects and covariates that were relevant for the respective traits; see Andersson-Eklund et al.<sup>4</sup> for a more detailed description of the statistical models used.

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5 Family was included to account for background genetic

effects and maternal effects. Carcass weight was included as a covariate to discern QTL effects on correlated traits, which means that all results concerning body composition were compared at equal weights. Least-squares 10 means for each genotype class at the IGF2 locus were estimated with a single point analysis using Procedure GLM of  $SAS^{29}$ ; the model included the same fixed effects and covariates as used in the interval mapping analyses. 15 The QTL shows a clear parent of origin-specific expression and the map position coincides with that of 10 the insulin-like growth factor II gene (IGF2), indicating IGF2 as the causative gene. A highly significant 20 segregation distortion (excess of Wild Boar-derived alleles) was also observed at this locus. The results 15 demonstrate an important effect of the IGF2 region on postnatal development and it is possible that the 25 presence of a paternally expressed IGF2-linked QTL in humans and in rodent model organisms has so far been overlooked due to experimental design or statistical 30 treatment of data. The study has also important implications for quantitative genetics theory and practical pig breeding. IGF2 was identified as a positional candidate gene 35 for this QTL due to the observed similarity between pig 25 chromosome 2p and human chromosome 11p. A genomic IGF2 clone was isolated by screening a porcine BAC library. FISH analysis with this BAC clone gave a strong 40 consistent signal on the terminal part of chromosome 2p (Fig. 1). A polymorphic microsatellite is located in the 30 3'UTR of IGF2 in mice (GenBank U71085), humans (GenBank 45

S62623), and horse (GenBank AF020598). The possible presence of a corresponding porcine microsatellite was investigated by direct sequencing of the 1GF2 3'UTR using the BAC clone. A complex microsatellite was identified about 800 bp downstream of the stop codon; a sequence comparison revealed that this microsatellite is identical

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to a previously described anonymous microsatellite,  $Swc9^6$ . PCR primers were designed and the microsatellite (IGF2ms) was found to be highly polymorphic with three different alleles among the two Wild Boar founders and another two among the eight Large White founders. IGF2ms was fully informative in the intercross as the breed of origin as well as the parent of origin could be determined with confidence for each allele in each  $F_2$  animal.

A linkage analysis using the intercross pedigree was carried out with IGF2ms and the microsatellites Sw2443, Sw2623, and Swr2516, all from the distal end of  $2p^7$ . IGF2 was firmly assigned to 2p by highly significant lod scores (e.g. Z=89.0,  $\theta=0.003$  against Swr2516). Multipoint analyses, including previously typed chromosome 2 markers $^8$ , revealed the following order of loci (sexaverage map distances in Kosambi cM): Sw2443/Swr2516-0.3-IGF2-14.9-Sw2623-10.3-Sw256. No recombinant was observed between Sw2443 and Swr2516, and the suggested proximal location of IGF2 in relation to these loci is based on a single recombinant giving a lod score support of 0.8 for the reported order. The most distal marker in our previous QTL study, Sw256, is located about 25 cM from the distal end of the linkage group.

QTL analyses of body composition, fatness, meat quality, and growth traits were carried out with the new chromosome 2 map using a statistical model testing for the possible presence of an imprinting effect as expected for IGF2. Clear evidence for a paternally expressed QTL located at the very distal tip of 2p was obtained (Fig. 2; Table 1). The QTL had very large effects on lean meat content in ham and explained an astonishing 30% of the residual phenotypic variance in the F<sub>2</sub> population. Large effects on the area of the longissumus dorsi muscle, on the weight of the heart, and on back-fat thickness

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(subcutaneous fat) were also noted. A moderate effect on one meat quality trait, reflectance value, was indicated. The QTL had no significant effect on abdominal fat, birth weight, growth, weight of liver, kidney, or spleen (data not shown). The Large White allele at this QTL was

associated with larger muscle mass and reduced back-fat thickness consistent with the difference between this breed and the Wild Boar population. The strong imprinting effect observed for all affected traits strongly suggests

a single causative locus. The pleiotropic effects on skeletal muscle mass and the size of the heart appear adaptive from a physiological point of view as a larger muscle mass requires a larger cardiac cutput. The clear paternal expression of this QTL is illustrated by the

least squares means which fall into two classes following the population origin of the paternally inherited allele (Table 1). It is worth noticing though that there was a non-significant trend towards less extreme values for the two heterozygous classes, in particular for the estimated effect on the area of longissimus dorsi. This may be due

to chance, but could have a biological explanation, e.g. that there is some expression of the maternally inherited allele or that there is a linked, non-imprinted QTL with minor effects on the traits in question.

The IGF2-linked QTL and the FAT1 QTL on chromosome 4

The IGF2-linked QTL and the FATI QTL on chromosome 4 1, 9 are by far the two loci with the largest effect on body composition and fatness segregating in this Wild Boar intercross. The IGF2 QTL controls primarily muscle mass whereas FAT1 has major effects on fat deposition including abdominal fat, a trait that was not affected by the IGF2 QTL (Fig. 2). No significant interaction between the two loci was indicated and they control a very large proportion of the residual phenotypic variance in the  $F_2$  generation. A model including both QTLs explains 33.1% of the variance for percentage lean meat in ham, 31.3% for

the variance for percentage lean meat in ham, 31.3% for the percentage of lean meat plus bone in back, and 26.2%

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for average back fat depth (compare with a model including only chromosome 2 effects, Table 1). The two QTLs must have played a major role in the response during selection for lean growth and muscle mass in the Large White domestic pig.

A highly significant segregation distortion was observed in the IGF2 region (excess of Wild Boar-derived alleles) as shown in Table 1 ( $\chi$ 2=11.7, d.f.=2; P=0.003). The frequency of Wild Boar-derived IGF2 alleles was 59% in contrast to the expected 50% and there was twice as many "Wild Boar" as "Large White" homozygotes. This deviation was observed with all three loci at the distal tip and is thus not due to typing errors. The effect was also observed with other loci but the degree of 15 distortion decreased as a function of the distance to the distal tip of the chromosome. Blood samples for DNA preparation were collected at 12 weeks of age and we are convinced that the deviation from expected Mendelian ratios was present at birth as the number of animals lost prior to blood sampling was not sufficient to cause a deviation of this magnitude. No other of the more than 250 loci analyzed in this pedigree show such a marked segregation distortion (L. Andersson, unpublished). The segregation distortion did not show an imprinting effect, as the frequencies of the two reciprocal types of heterozygotes were identical (Table 1). This does not exclude the possibility that the QTL effects and the segregation distortion are controlled by the same locus. The segregation distortion maybe due to meiotic drive favoring the paternally expressed allele during gametogenesis, as the F1 parents were all sired by Wild Boar males. Another possibility is that the segregation distortion may be due to codominant expression of the maternal and paternal allele in some tissues and/or

35 during a critical period of embryo development. Biallelic

IGF2 expression has been reported to occur to some extent

during human development<sup>10</sup>, 11 and interestingly a strong influence of the parental species background on *IGF2* expression was recently found in a cross between *Mus musc*ulus and *Mus spretus*<sup>12</sup>. It is also interesting that a VNTR polymorphism at the insulin gene, which is very closely linked to *IGF2*, is associated with size at birth in humans<sup>13</sup>. It is possible that the *IGF2*-linked QTL in pigs has a minor effect on birth weight but in our data it was far from significant (Fig. 2) and there was no indication of an imprinting effect.

This study is an advance in the general knowledge concerning the biological importance of the IGF2 locus. The important role of IGF2 for prenatal development is well-documented from knock-out  $mice^{14}$  as well as from its causative role in the human Beckwith-Wiedemann syndrome<sup>15</sup>. This study demonstrates an important role for the IGF2-region also for postnatal development. It should be stressed that our intercross between outbred populations is particularly powerful to detect QTL with a parent of origin-specific effect on a multifactorial trait. This is because multiple alleles (or haplotypes) are segregating and we could deduce whether a heterozygous  $F_2$  animal received the Wild Boar allele from the  $F_1$  male or female. It is quite possible that the segregation of a paternally expressed IGF2-linked QTL affecting a trait like obesity has been overlooked in human studies or in intercrosses between inbred rodent populations because of experimental design or statistical treatment of data. An imprinting effect cannot be detected in an intercross between two inbred lines as only two alleles are segregating at each locus. Our result has therefore significant bearings on the future analysis of the association between genetic polymorphism

obesity $^{17}$ , and variation in birth weight $^{13}$  in humans, as

in the insulin-IGF2 region and Type I diabetes  $^{16}$ ,

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well as for the genetic dissection of complex traits using inbred rodent models. A major impetus for generating an intercross between the domestic pig and its wild ancestor was to explore the possibilities to map and identify major loci that have responded to selection. We 10 have now showed that two single QTLs on chromosome 2 (this study) and  $4^{1}$ , 2 explain as much as one third of the phenotypic variance for lean meat content in the  $F_2$ 15 generation. This is a gross deviation from the underlying assumption in the classical infinitesimal model in quantitative genetics theory namely that quantitative traits are controlled by an infinite number of loci each 20 with an infinitesimal effect. If a large proportion of the genetic difference between two divergent populations (e.g. Wild Boar and Large White) is controlled by a few loci, one would assume that selection would quickly fix 25 QTL alleles with large effects leading to a selection plateau. However, this is not the experience in animal breeding programs or selection experiments where good persistent long-term selection responses are generally 30 obtained, provided that the effective population size is reasonably large 18. A possible explanation for this paradox is that QTL alleles controlling a large 35 proportion of genetic differences between two populations 25 may be due to several consecutive mutations; this may be mutations in the same gene or at several closely linked genes affecting the same trait. It has been argued that 40 new mutations contribute substantially to long-term selection responses  $^{19}$ , but the genomic distribution of such mutations are unknown. 30 The search for a single causative mutation is the 45 paradigm as regards the analysis of genetic defects in mice and monogenic disorders in humans. We propose that this may not be the case for loci that have been under selection for a large number of generations in domestic. 50 animals, crops, or natural populations. This hypothesis

predicts the presence of multiple alleles at major QTL. It gains some support from our recent characterization of porcine coat color variation. We have found that both the alleles for dominant white color and for black-spotting 10 5 differ from the corresponding wild-type alleles by at least two consecutive mutations with phenotypic effects at the KIT and MCIR loci, respectively 20, 21. In this context it is highly interesting that in the accompanying 15 example we have identified a third allele at the IGF2linked QTL. The effects on muscle mass of the three alleles rank in the same order as the breeds in which they are found i.e. Piétrain pigs are more muscular than 20 Large White pigs that in turn have higher lean meat

content than Wild Boars.

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There are good reasons to decide that IGF2 is the causative gene for the now reported QTL. Firstly, there is a perfect agreement in map localization (Fig. 2). Secondly, it has been shown that IGF2 is paternally expressed in mice, humans, and now in pigs, like the QTL. There are several other imprinted genes in the near vicinity of IGF2 in mice and humans (Mash2, INS2, H19, KVLQT1, TAPA1/CD81, and CDKN1C/p57KIP2) but only IGF2 is paternally expressed in adult tissues<sup>22</sup>. We believe that this locus provides a unique opportunity for molecular characterization of a QTL. The clear paternal expression can be used to exclude genes that do not show this mode of inheritance. Moreover, the presence of an allelic series should facilitate the difficult distinction between causative mutations and linked neutral polymorphism. We have already shown that there is no difference in coding sequence between IGF2 alleles from Piétrain and Large White pigs suggesting that the causative mutations occur in regulatory sequences. An obvious step is to sequence the entire IGF2 gene and its

multiple promoters from the three populations. The recent

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report that a VNTR polymorphism in the promoter region of the insulin (INS) gene affects IGF2 expression<sup>23</sup> suggests that the causative mutations may be at a considerable distance from the IGF2 coding sequence.

The results have several important implications for the pig breeding industry. They show that genetic imprinting is not an esoteric academic question but need

White, and Piétrain populations indicates that further alleles at the IGF2-linked QTL segregate within commercial populations. The paternal expression of the QTL facilitates its detection using large paternal half-sib families as the female contribution can be ignored.

to be considered in practical breeding programs. The

detection of three different alleles in Wild Boar, Large

15 The QTL is exploited to improve lean meat content by marker assisted selection within populations or by marker assisted introgression of favorable alleles from one population to another.

Example 2: Piétrain x Large White intercrosses

## Methods

Pedigree material: The pedigree material utilized to map

5 QTL was selected from a previously described Piétrain x
Large White F2 pedigree comprising > 1,800 individuals<sup>6,7</sup>.

To assemble this F2 material, 27 Piétrain boars were
mated to 20 Large White sows to generate an F1 generation
comprising 456 individuals. 31 F1 boars were mated to

0 unrelated 82 F1 sows from 1984 to 1989, yielding a total

- of 1862 F2 offspring. F1 boars were mated on average to 7 females, and F1 sows to an average of 2,7 males. Average offspring per boar were 60 and per sow 23.
- Phenotypic information: (i) Data collection: A total of 21 distinct phenotypes were recorded in the F2 generation<sup>6.7</sup>. These included:
  - five growth traits: birth weight (g), weaning weight (Kg), grower weight (Kg), finisher weight (Kg) and
- 20 average daily gain (ADG; Kg/day; grower to finsher period);
  - two body proportion measurements: carcass length (cm); and a conformation score (0 to 10 scale; ref.6);
  - ten measurements of carcass composition obtained by
- dissection of the chilled carcasses 24 hours after slaughter. These include measurements of muscularity: % ham (weight hams/carcass weight), % loin (weight loin/carcass weight), % shoulder ( weight
- shoulder/carcass weight), % lean cuts (% ham + %loin + % 30 shoulder); and measurements of fatness: average back-fat thickness (BFT; cm), % backfat (weight backfat/carcass.
  - weight), % belly (weight belly/carcass weight), % leaf
    fat (weight leaf fat/carcass weight), % jowl (weight
    jowl/carcass weight), and "% fat cuts" (% backfat + %
- 35 belly + % leaft fat + % jowl).
  - four meat quality measurements: pH  $_{
    m LD1}$  (Longissimus dorsi 1

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CCTGAGCTGCAGCAGGCCAG-3').

hour after slaughter), pH <sub>tD24</sub> (*Longissimus dorsi* 24 hours after slaughter), pH <sub>G1</sub> (*Gracilis* 1 hour after slaughter) and pH <sub>G24</sub> (*Gracilis*. 24 hours after slaughter). (ii) Data processing: Individual phenotypes were preadjusted for fixed effects (sire, dam, CRC genotype, sex, year-season, parity) and covariates (litter size, birth weight, weaning weight, grower weight, finisher weight) that proved to significantly affect the corresponding trait. Variables included in the model were selected by stepwise regression.

Marker genotyping: Primer pairs utilized for PCR amplification of microsatellite markers are as described<sup>19</sup>. Marker genotyping was performed as previously described<sup>20</sup>. Genotypes at the CRC and MyoD loci were determined using conventional methods as described<sup>1,12</sup>. The LAR test for the Igf2 SNP was developed according to Baron et al.<sup>21</sup> using a primer pair for PCR amplification (5'-CCCCTGAACTTGAGGACGAGCC-3';5'-ATCGCTGTGGGCTGGGTGGCCGCC-3') and a set of three primers for the LAR step (5'-FAM-CGCCCCAGCTGCCCCCCAA-3'; 5'-HEX-CGCCCCAGCTGCCCCCAA-3'; 5'-

Map construction: Marker maps were constructed using the TWOPOINT, BUILD and CHROMPIC options of the CRIMAP package<sup>22</sup>. To allow utilisation of this package, full-sib families related via the boar or sow were disconnected and treated independently. By doing so, some potentially usable information was neglected, yielding, however, unbiased estimates of recombination rates.

QTL mapping: (i) Mapping Mendelian QTL: Conventional QTL mapping was performed using a multipoint maximum likelihood method. The applied model assumed one segregating QTL per

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chromosome, and fixation of alternate QTL alleles in the respective parental lines, Piétrain (P) and Large White (LW). A specific analysis program had to be developed to account for the missing genotypes of the parental generation, resulting in the fact that the parental origin of the F1 chromosomes could not be determined. Using a typical "interval mapping" strategy, an hypothetical QTL was moved along the marker map using user-defined steps. At each position, the likelihood (L) of the pedigree data was

10 ·computed as:

$$L = \sum_{n=1}^{2'} \prod_{i=1}^{n} \sum_{G \in I}^{4} (P(G|M_i, \theta, \varphi)P(y_i|G))$$

P or right chromosme P), there is a total of  $2^{r}$  combinations for r F1 parents.

∏ n F2

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 $\sum_{i=1}^{4}$  ith F2 offspring, over the four possible QTL genotypes:

P/P, P/LW, LW/P and LW/LW

 $P(G|M_i,\theta,\varphi)M_i$ : the marker genotype of the ith F2 offspring and its F1 parents, (ii) : the vector of recombination rates between adjacent markers and between the hypothetical QTL and its flanking markers, and (iii) $\theta$  the considered marker-QTL phase combination of the F1 parents.

Recombination rates and marker linkage phase of F1 parents are assumed to be known when computing this probability. Both were determined using CRIMAP in the map construction phase (see above).

 $P(y_i|G)y_i)$  of offspring i, given the QTL genotype under consideration. This probability is computed from the normal density function:

$$P(y_i|G) = \frac{1}{\sqrt{2\pi}\sigma} e^{\frac{-(y_i - \mu_G)^2}{2\sigma^2}}$$

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 $_{G}$  is the phenotypic mean of the considered QTL genotype (PP, PL, LP or LL) and  $\sigma^{2}$  the residual variance  $\sigma^{2}$  was considered to be the same for the four QTL genotypic classes.

The values of  $\mu_{PP}$ ,  $\mu_{PL}=\mu_{LP}$ ,  $\mu_{LL}$  and  $\sigma^2$  maximizing L were determined using the GEMINI optimisation routine<sup>23</sup>. The likelihood obtained under this alternative  $H_1$  hypothesis was compared with the likelihood obtained under the null hypothesis  $H_0$  of no QTL, in which the phenotypic means of the four QTL genotypic classes were forced to be identical. The

four QTL genotypic classes were forced to be identical. The difference between the logarithms of the corresponding likelihoods yields a lodscore measuring the evidence in favour of a QTL at the corresponding map position.

(ii) Significance thresholds: Following Lander & Botstein<sup>24</sup>,

lodscore thresholds (T) associated with a chosen genome-wise significance level, were computed such that:

 $\alpha = (C + 9.21GT)\chi_2^2(4.6T)$ 

 ${\it C}$  corresponds to the number of chromosomes (= 19),  ${\it G}$  corresponds to the length of the genome in Morgans (= 29),

and  $\chi^2_2$  (4.6T) denotes one minus the cumulative distribution function of the chi-squared distribution with 2 c.f. Single point 2ln(LR) were assumed to be distributed as a chi-squared distribution with two degrees of freedom, as we were fitting both an additive and dominance component. To account for the

fact that we were analysing multiple traits, significance levels were adjusted by applying a Bonferoni correction corresponding to the effective number of independent traits that were analyzed. This effective number was estimated at 16 following the approach described by Spelman et al.<sup>25</sup>.

30 Altogether, this allowed us to set the lodscore threshold associated with an experiment-wise significance level of 5%

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at 5.8. When attempting to confirm the identified QTL in an independent sample, the same approach was used, however, setting C at 1, G at 25cM and correcting for the analysis of 4.5 independent traits (as only six traits were analyzed in this sample). This yielded a lodscore threshold associated with a Type I error of 5% of 2. (iii). Testing for an imprinted QTL: To test for an imprinted QTL, we assumed that only the QTL alleles transmitted by the parent of a given sex would have an effect on phenotype, the QTL alleles transmitted by the other parent being "neutral". The likelihood of the pedigree data under this hypothesis was computed using equation 1. To compute  $P(y_i \mid G)$ , however, the phenotypic means of the four QTL genotypes were set at  $\mu_{PP}$  =  $\mu_{PL}$  =  $\mu_{P}$  and  $\mu_{LP}$  =  $\mu_{LL}$  =  $\mu_{L}$  to test for a QTL for which the paternal allele only is expressed, and  $\mu_{PP}$  =  $\mu_{LP}$  =  $\mu_P$  and  $\mu_{PL}$  =  $\mu_{\text{LL}}$  =  $\mu_{\text{L}}$  to test for a QTL for which the maternal allele only is expressed. It is assumed in this notation that the first subscript refers to the paternal allele, the second subscript to the maternal allele.  ${
m H_0}$  was defined as the null-hypothesis of no QTL,  $\ensuremath{\text{H}_{1}}$  testing the presence of a Mendelian QTL;  $\ensuremath{\text{H}_{2}}$ 20 testing the presence of a paternally expressed QTL, and  $\ensuremath{\text{H}}_3$ testing the presence of a maternally expressed QTL.

RT-PCR: Total RNA was extracted from skeletal muscle according to Chirgwin et al. 26. RT-PCR was performed using the Gene-Amp RNA PCR Kit (Perkin-Elmer) The PCR products were purified using QiaQuick PCR Purification kit (Qiagen) and sequenced using Dye terminator Cycle Sequencing Ready Reaction (Perkin Elmer) and an ABI373 automatic sequencer.

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In example 2 we report the identification of a QTL with major effect on muscle mass and fat deposition mapping to porcine 2p1.7 The QTL shows clear evidence for parental imprinting strongly suggesting the involvement of the *Iqf2* locus.

offspring was generated as described<sup>6,7</sup>. The Large White and Piétrain parental breeds differ for a number of economically important phenotypes. Piétrains are famed for their exceptional muscularity and leannes <sup>8</sup> (Figure 2), while Large Whites show superior growth performance. Twenty-one distinct phenotypes measuring (i) growth performance (5), (ii) muscularity (6), (iii) fat deposition (6), and (iv) meat quality (4), were recorded on all F<sub>2</sub> offspring.

In order to map QTL underlying the genetic differences between these breeds, we undertook a whole genome scan using microsatellite markers on an initial sample of 677 F2 individuals. Analysis of pig chromosome 2 using a ML multipoint algorithm, revealed highly significant lodscores (up to 20) for six of the 12 phenotypes measuring muscularity and fat deposition at the distal end of the short arm of chromosome 2 (Figure 3a). Positive lodscores were obtained for the remaining six phenotypes, however, not reaching the genome-wise significance threshold ( = 5%). To confirm this finding, the remaining sample of 355  $F_2$  offspring was genotyped for the five most distal 2p markers and QTL analysis performed for the traits yielding the highest lodscores in the first analysis. Lodscores ranged from 2.1 to 7.7, clearly confirming the presence of a major QTL in this region. Table 2 reports the corresponding ML estimates for the three genotypic means as well as the corresponding residual variance.

Bidirectional chromosome painting establishes a correspondence between SSC2p and HSAllpter-q13<sup>9,10</sup>. At least

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two serious candidate genes map to this region in man: the myogenic basic helix-loop-helix factor, MyoD, maps to HSA11p15.4, while Igf2 maps to HSA11p15.5 MyoD is a well known key regulator of myogenesis and is one of the first myogenic markers to be switched on during development11. A previously described amplified sequence polymorphism in the porcine MyoD gene12 proved to segregate in our F2 material, which was entirely genotyped for this marker. Linkage analysis positioned the MyoD gene in the SW240-SW776 (odds > 1000) interval, therefore well outside the lod-2 drop off support interval for the QTL (figure 1). Igf2 is known to enhance both proliferation and differentiation of myoblasts in vitro13 and to cause a muscular hypertrophy when overexpressed in vivo. Based on a published porcine adult liver cDNA sequence 14, we designed primer pairs allowing us to amplify the entire Igf2 coding sequence with 222 bp of leader and 280 bp of trailor sequence from adult skeletal muscle cDNA. Piétrain and Large White RT-PCR products were sequenced indicating that the coding sequences was identical in both breeds and with the published sequence. However, a G A transition was found in the leader sequence corresponding to excn 2 in man (Figure 4). We developed a screening test for this single nucleotide polymorphism (SNP) based on the ligation amplification reaction (LAR), allowing us to genotype our pedigree material. Based on these data, Igf2 was shown to colocalize with the SWC9 microsatellite marker ( = 0%), therefore located at approximately 2 centimorgan from the most likely position of the QTL and well within the 95% support interval for the QTL (figure 1). Subsequent sequence analysis demonstrated that the microsatellite marker SWC9 is actually located within the 3' UTR of the Igf2 gene. Combined with available comparative mapping data for the PGA and FSH loci, these results suggest the occurrence of an interstitial

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inversion of a chromosome segment containing MyoD, but not Iqf2 which has remained telomeric in both species.

Igf2 therefore appeared as a strong positional allele having the observed QTL effect. In man and mouse, Iqf2 is known to be imprinted and to be expressed exclusively from the paternal allele in several tissues15. Analysis of skeletal muscle cDNA from pigs heterozygous for the SNP and/or SWC9, shows that the same imprinting holds in this tissue in the pig as well (Figure 4). Therefore if Igf2 were 10 · responsible for the observed effect, and knowing that only the paternal Igf2 allele is expressed, one can predict that (i) the paternal allele transmitted by F1 boars (P or LW) would have an effect on phenotype of F2 offspring, (ii) the maternal allele transmitted by Fl sows (P or LW) would have no effect on phenotype of F2 offspring, and (iii) the likelihood of the data would be superior under a model of a bimodal (1:1) F2 population sorted by inherited paternal allele when compared to a conventional "Mendelian" model of a trimodal (1:2:1) F2 population. The QTL mapping programs were adapted in order to allow testing of the corresponding hypotheses. Ho was defined as the null-hypothesis of no QTL,  $H_1$  as testing for the presence of a Mendelian QTL,  $H_2$  as testing for the presence of a paternally expressed QTL, and H<sub>3</sub> as testing for the presence of a maternally expressed QTL.

Figure 3 summarizes the obtained results. Figure 3a, 3b and 3c respectively show the lodscore curves corresponding to  $\log_{10}~(\mathrm{H_2/H_0})$ ,  $\log_{10}~(\mathrm{H_3/H_0})$  and  $\log_{10}~(\mathrm{H_2/H_1})$ . It can be seen that very significant lodscores are obtained when testing for the presence of a paternally expressed QTL, while there is no evidence at all for the segregation of a QTL when studying the chromosomes transmitted by the sows. Also, the hypothesis of a paternally expressed QTL is significantly more likely (  $\log_{10}~(\mathrm{H_2/H_1})~>~3$ ) than the hypothesis of a "Mendelian" QTL

for all examined traits. The fact that the same tendency is observed for all traits indicates that it is likely the same imprinted gene that is responsible for the effects observed on the different traits. Table 2 reports the ML phenotypic means for the F2 offspring sorted by inherited paternal QTL allele. Note that when performing the analysis under a model of a mendelian QTL, the Piétrain and Large White QTL alleles appeared to behave in an additive fashion, the heterozygous genotype exhibiting a phenotypic mean corresponding exactly to the midpoint between the two homzygous genotypes. This is exactly what one would predict when dealing with an imprinted QTL as halve of the heterozygous offspring are expected to have inherited the P allele from their sire, the other halve the LW allele.

These data therefore confirmed our hypothesis of the involvement of an imprinted gene expressed exclusively from the paternal allele. The fact that the identified chromosomal segment coincides precisely with an imprinted domain documented in man and mice strongly implicates the orthologous region in pigs. At least seven imprinted genes mapping to this domain have been documented (Igf2, Ins2, H19, Mash2, p57<sup>K1P2</sup>, K<sub>V</sub>LQTL1 and TDAG51) (ref. 15 and Andrew

Feinberg, personal communication). Amongst these, only *Igf2* and *Ins2* are paternally expressed. While we cannot exclude that the observed QTL effect is due to an as of yet unidentified imprinted gene in this region, its reported effects on myogenesis *in vitro* and *in vivo*<sup>13</sup> strongly

implicate *Igf2*. Particularly the muscular hypertrophy observed in transgenic mice overexpressing *Igf2* from a muscle specific promotor are in support of this hypothesis (Nadia Rosenthal, personal communication. Note that allelic variants of the *INS* VNTR have recently been shown to be associated

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with size at birth in  $man^{16}$ , and that the same VNTR has been shown to affect the level of Igf2 expression<sup>17</sup>.

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The observation of the same QTL effect in a Large White x Wild Boar intercross indicates the existence of a series of at least three distinct functional alleles. Moreover, preliminary evidence based on marker assisted segregation analysis points towards residual segregation at this locus within the Piétrain population (data not shown). The occurrence of an allelic series might be invaluable in identifying the causal polymorphisms which - based on the quantitatve nature of the observed effect - are unlikely to be gross gene alterations but rather subtle regulatory

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The effects of the identified QTL on muscle mass and fat deposition are truly major, being of the same magnitude of those reported for the CRC locus<sup>6,7</sup> though apparently without the associated deleterious effects on meat quality. We estimate that both loci jointly explain close to 50% of the Piétrain versus Large White breed difference for muscularity and leanness. Understanding the parent-of-origin effect characterizing this locus will allow for its optimal use in breeding programs. Indeed, today half of the offspring from commercially popular Piétrain x Large White crossbred boars inherit the unfavourable Large White allele causing

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25 considerable loss.

mutations.

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The QTL described in this work is the second example of a gene affecting muscle development in livestock species that exhibits a non-mendelian inheritance pattern. Indeed, we have previously shown that the callipyge locus (related to the qualitative trait wherein muscles are doubled) is characterized by polar overdominance in which only the heterozygous individuals that inherit the CLPG mutation from their sire express the double-muscling phenotype<sup>5</sup>. This

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demonstrates that parent-of-origin effects affecting genes underlying production traits in livestock might be relatively common.

Example 3:

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Generating a reference sequence of IGF2 and flanking loci in the pig.

10 The invention provides an imprinted QTL with major effect on muscle mass mapping to the IGF2 locus in the pig, and use of the QTL as tool in marker assisted selection. To fine tune this tool for marker assisted selection, as well as to further identify a causal mutation, we have further generated a reference sequence encompassing the entire porcine IGF2 sequence as well as that from flanking genes.

To achieve this, we screened a porcine BAC library with IGF2 probes and identified two BACs. BAC-PIGF2-1 proved to 20 contain the INS and IGF2 genes, while BAC-PIGF2-2 proved to contain the IGF2 and H19 genes. The NotI map as well as the relative position of the two BACs is shown in Figure 5. BAC-PIGF2-1 was shorgun sequenced using standard procedures and automatic sequencers. The resulting sequences were assembled using standard software yielding a total of 115 contigs. The corresponding sequences are reported in figure 6. Similarity searches were performed between the porcine contigs and the orthologous sequences in human. Significant homologies were

For BAC-PIGF2-2, the 24 Kb NotI fragment not present in BAC-PIGF2-1 was subcloned and sequenced using the EZ::TN transposon approach and ABI automatic sequencers. Resulting

detected for 18 contigs and are reported in Figure 7.

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; ;	43	,
7 <b>0</b> 5	sequences were assembled using the Phred-Phra suit, yielding seven distinct contigs (figure sequences were aligned with the corresponding human sequences using the compare and dotplot GCG suite. Figure 9 symmarizes the corresponding	8). The contig orthologous programs of the
15	Example 4: Identification of DNA sequence polthe IGF2 and flanking loci.	ymorphisms in
10	Example 1, we resequenced part of the IGF2 and from genomic DNA isolated from Pietrain, Larg	d flanking loci e White and Wild
25	Boar individuals, allowing identification of polymorphisms such as reported in figure 10.	DNA sequence
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Legends to the figures

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Fig. 1: Test statistic curves obtained in QTL analyses of

5 chromosome 2 in a Wild Boar/Large White intercross. The graph
plots the F ratio testing the hypothesis of a single QTL at a
given position along the chromosome for the traits indicated.
The marker map with the distances between markers in Kosambi
centiMorgan is given on the X-axis. The horizontal lines

10 represent genome-wise significant (P<0.05) and suggestive
levels for the trait lean meat in ham; similar significance
thresholds were obtained for the other traits.

20

Figure 2: Piétrain pig with characteristic muscular hypertrophy.

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Figure 3: Lodscore curves obtained in a Piétrain x Large White intercross for six phenotypes measuring muscle mass and fat deposition on pig chromosome 2. The most likely positions

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of the *Igf2* and *MyoD* genes determined by linkage analysis with respect to the microsatellite marker map are shown. Ho was defined as the null-hypothesis of no QTL, H<sub>1</sub> as testing for the presence of a Mendelian QTL, H<sub>2</sub> as testing for the presence of a paternally expressed QTL, and H<sub>3</sub> as testing for

35

25 the presence of a maternally expressed QTL. 3a:  $log_{10}\left(H_1/H_{01}, 3b: log_{10}\left(H_2/H_0\right), 3c: log_{10}\left(H_3/H_0\right)\right)$ 

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Figure 4: A. Structure of the human *Igf2* gene according to ref. 17, with aligned porcine adult liver cDNA sequence as reported in ref. 16. The position of the *nt241(G-A)* transition and *Swc9* microsatellite are shown. B. The corresponding markers were used to demonstrate the monoallelic (paternal) expression of *Igf2* in skeletal muscle

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5 and liver of 10-week old fetuses. PCR amplification of the nt421(G-A) polymorphism and Swc9 microsatellite from genomic DNA clearly shows the heterozygosity of the fetus, while only 10 the paternal allele is detected in liver cDNA (nt421(G-A) and 5 Swc9) and muscle cDNA (Swc9). The absence of RT-PCR product for nt421(G-A) from in fetal muscle points towards the absence of mRNA including exon 2 in this tissue. Parental 15 origin of the foetal alleles was determined from the genotypes of sire and dam (data not shown). 10 . 20 Figure 5: A NotI restriction map showing the relative position of BAC-PIGF2-1 (comprising INS and IGF2 genes), and BAC-PIGF2-2 (comprising IGF2 and H19 genes). 25 15 Figure 6: Nucleic acid sequences of contig 1 to contig 115 derived from BAC-PIGF2-1 which was shotgun sequenced using standard procedures and automatic sequencers. 30 Figure 7: Similarity between porcine contigs of figure 6 and orthologous sequences in human. Figure 8 Nucleic acid sequences of contig 1 to contig 7 35 derived from BAC-PIGF2-2, (the 24 Kb NotI fragment not present in BAC-PIGF2-1) which was subcloned and sequenced using the EZ::TN transposon approach and ABI automatic 40 sequencers. Figure 9: Similarity between porcine contigs of figure 8 and orthologous sequences in human. 45

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Figure 10: DNA sequence polymorphisms in the IGF2 and flanking loci from genomic DNA isolated from Piétrain, Large White and Wild Boar individuals.

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		wo	00/3614	3							52				PC	T/EP9	9/1020	9
5	•	-		•	n=30		67.3	5.02₺	70.8°	35.2 <sup>b</sup>	-	24.7	244 <sup>b</sup>	19.7				
10			NW/47		n=43		66.4°	4.94b	69.3	34.5		25.5 <sup>b</sup>	238 <sup>b</sup>	21.8b				
15	·¥		Least squares means' WP/WM WP/LM		n=43		64.2	4.72*	66.7*	33.0		27.7°	225*	18.4				ē
		.S.	Least squ		n=62		63.6	4.69	66.3	31.9		27.2*	226	18.6				•
20	•	ercros						٠,										
-		ite int	of F,															
25		r/Large Wh	Percent of F,				30.6	24.3	17.4	15.4		10.4	14.4					
30		a Wild Boar	Map position <sup>3</sup>				0		0	-		0	. 0					
		mosome 2 in	Imprinting				19.1***	16.8***	**9.6	*8.4		8.7**	11.4***	<b>*</b> 1. <b>9</b>				
35		is for ple chro	F ratio <sup>2</sup> OTL	)			24.4***	18.1***	12.2**	10.3**		7.1*	9.7**	7.5				
40		of OTL analys	-			aits	%	nam, kg	n back, %	e area, cm²		depth, mm	rgans		***P<0.001			
<b>45</b>		Table I Summary of OTL analysis for pig chromosome 2 in a Wild Boar/Large White intercross.	Trait	$\Gamma_{P/L^M}$	-,	Body composition traits	Lean meat in ham, %	Lean meat mass in ham, kg	Lean meat + bone in back, %	Longissimus muscle area, cm²	Fatness traits	Average back fat de	Weight of internal organs Heart, gram	<u>Meat quality traits</u> Reflectance value FEL	*P<0.05; **P<0.01; ***P<0.001			
50		•			ß	щ			10			15	.,	20	-			

Table 1, continued

'Only the traits for which the QTL peak was in the IGF2

region (0-10 cM) and the test statistic reached the nominal significance threshold of F=3.9 are included.

""QTL" is the test statistic for the presence of a QTL under a genetic model with additive, dominance, and imprinting effects (3 d.f.) while "Imprinting" is the test statistic for the presence of an imprinting effect (1 d.f.), both obtained at the position of the QTL peak. Genome-wise significance thresholds, estimated by permutation, were used for the QTL test while nominal significance thresholds were used for the Imprinting test.

<sup>3</sup>In cM from the distal end of 2p; IGF2 is located at 0.3 cM.
<sup>4</sup>The reduction in the residual variance of the F<sub>2</sub> population effected by inclusion of an imprinted QTL at the given position.

SMeans and standard errors estimated at the IGF2 locus by

classifying the genotypes according to the population and
parent of origin of each allele. W and L represent alleles
derived from the Wild Boar and Large White founders,
respectively; superscript P and M represent a paternal and
maternal origin, respectively. Figures with different letters

(superscript a or b) are significantly different at least at
the 5% level, most of them are different at the 1% or 0.1%

level.

Table 2 Maximum likelihood phenotypic means for the different F2 genotypes estimated under (i) a model of a mendelian QTL, and (ii) a model assuming an imprinted QTL.

	Mendeli	an QTL			Imprin	ted QT	L
Traits							
	hrm/rm	HEM/P	.h6/5	Ř	PPAT/LW	UPAT/P	R
BFT (cm)	2.98	2.84	2.64	0.27	2.94	2.70	0.27
% ham	21.10	21.56	22.15	0.83	21.23	21.9	0.83
						5	
% loin	24.96	25.53	26.46	0.91	25.12	26.1	0.93
						4	
% lean	65.02	65.96	67.60	1.65	65.23	67.0	1.67
cuts		·				5	
g	6.56	6.02	5.33	0.85	6.43	5.56	0.85
backfat							
% fat	28.92	27.68	26.66	1.46	28.54	26.9	1.49
cuts						9	

## Claims

CLAIMS 10 1. A method for selecting a domestic animal for having desired genotypic properties comprising testing said animal for the presence of a parentally imprinted quantitative trait 15 locus (QTL). 5 2. A method according to claim 1 further comprising testing a nucleic acid sample from said animal for the presence of a parentally imprinted quantitative trait locus (QTL). 20 3. A method according to claim 1 or 2 wherein in the pig said OTL is located at chromosome 2. 10 4. A method according to claim 2 or 3 wherein said QTL is mapping at around position 2p1.7. 25 5. A method according to claim 1 to 4 wherein said QTL is related to the potential muscle mass and/or fat deposition of said animal. 6. A method according to claim 5 wherein said QTL comprises at least a part of an insulin-like growth factor-2 (IGF2) 30 gene. 7. A method according to anyone of claims 1 to 6 wherein in the pig said QTL comprises a marker characterised as nt241(G-20 A) or as Swc9, as identified in figure 4. 35 8. A method according to anyone of claims 1-7 wherein a paternal allele of said QTL is predominantly expressed in said arimal. 9. A method according to anyone of claims 1-7 wherein a 40 maternal allele of said QTL is predominantly expressed in said animal. 10. An isolated and/or recombinant nucleic acid comprising a parentally imprinted quantitative trait locus (QTL) or. 45 functional fragment derived thereof. 30 11. An isolated and/or recombinant nucleic acid comprising a synthetic parentally imprinted quantitative trait locus (QTL)

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5		56
	derived from a	least one chromosome or functional fragment
10	derived thereo	
	12. A nucleic	acid according to claim 10 or 11 at least
		from a Sus scrofa chromosome.
15		acid according to claim 12 wherein said nucleio
		st partly derived from a Sus scrofa chromosome
		from a region mapping at around position 2p1.7
	14. A nucleic.	acid according to any one of claims 10 to 13
	wherein said Q	TL is related to the potential muscle mass
		osition of said animal.
20		acid according to any one of claims 10 to 14
	wherein said Q	TL comprises at least a part of a insulin-like
	growth factor-	
		acid according to anyone of claims 10 to 15
25	15 wherein a pate	rnal allele of said QTL is capable of being
	predominantly	
		acid according to anyone of claims 10 to 16
		rnal allele of said QTL is capable of being
30	predominantly	
		ucleic acid or fragment derived thereof
	according to c	laim 10 in a method according to anyone of
	claims 1-9.	
35		ing to claim 18 to select a breeding animal or
		d for slaughter for having desired genotypic o
		otypic properties.
		ing to claim 19 wherein said properties are
40		cle mass and/or fat deposition.
•	21. An animal	such as pig selected by a use according to
	claim 18 to 20	
		ccording to claim 21 characterised in being
45 ·		an allele at a paternally imprinted QTL,
	preferably loc	ared at a Sus scrofa chromosome 2 mapping at

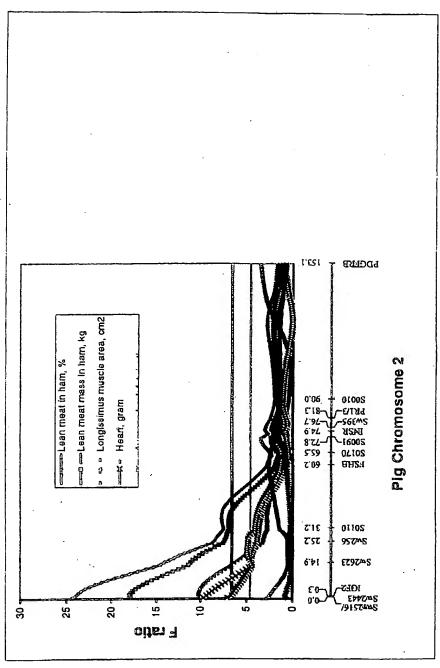
23. An animal according to claim 21 or 22 wherein said QTL is related to the potential muscle mass and/or fat deposition of

around position 2p1.7.

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	11 0 000001.10	
5		57
. 10	a insuli 24. A ti	g and/or wherein said QTL comprises at least a part of n-like growth factor-2 (IGF2) allele. cansgenic animal comprising a nucleic acid according see of claims 11 to 16.
15	male. 26. Sper	mimal according to anyone of claims 21-24 which is a mor an embryo derived from an animal according to of claims 21-25.
20		of a sperm or an embryo according to claim 26 in animals destined for slaughter.
25		
30		

FIGURE 1

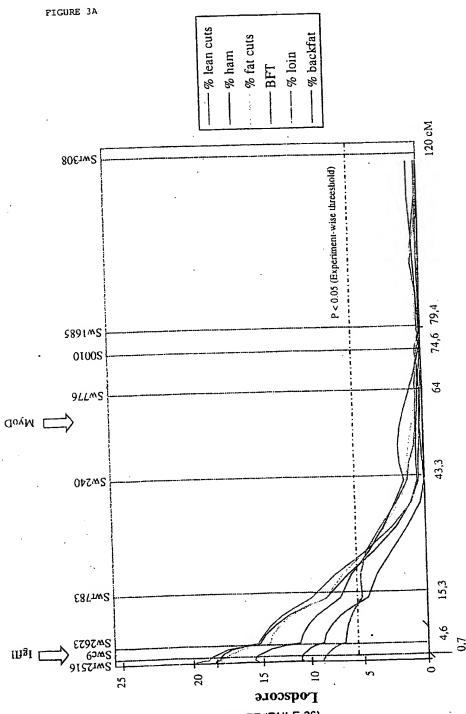


SUBSTITUTE SHEET (RULE 26)

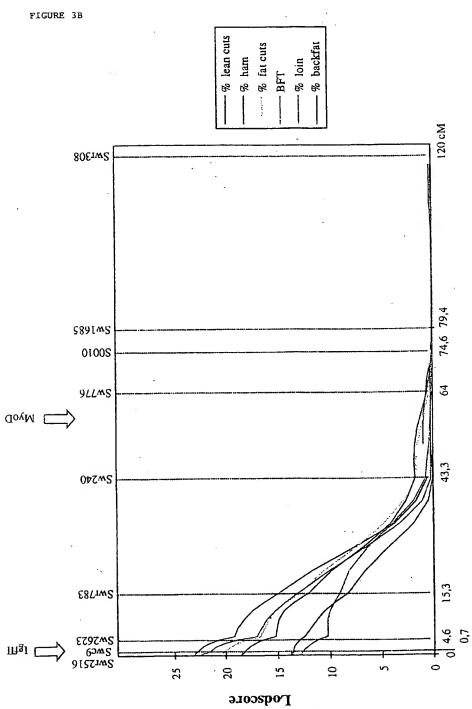
FIGURE 2



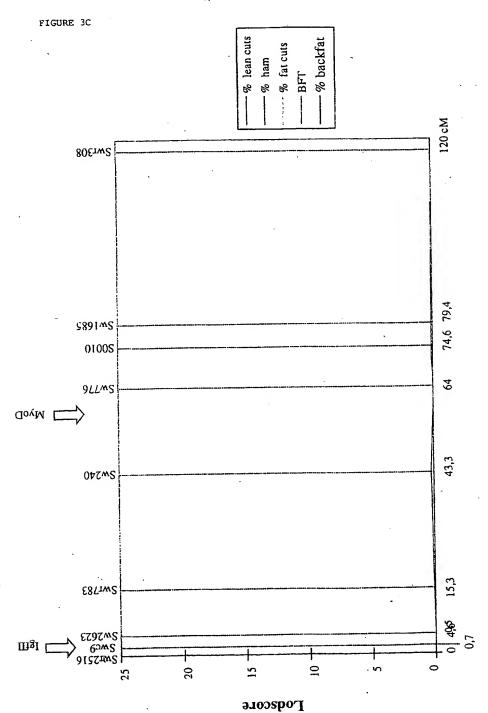
SUBSTITUTE SHEET (RULE 26)



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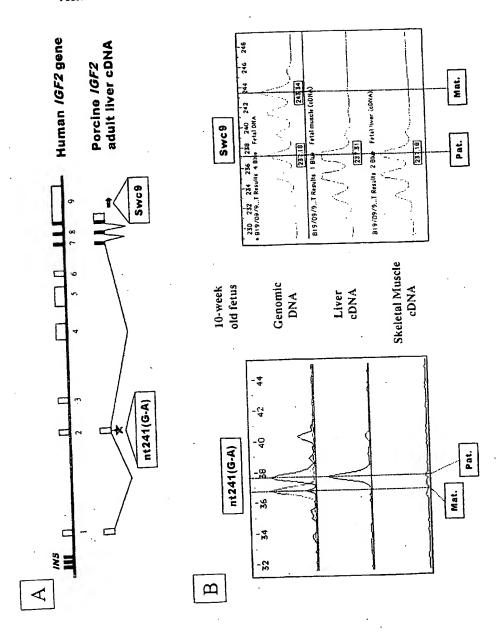


SUBSTITUTE SHEET (RULE 26)



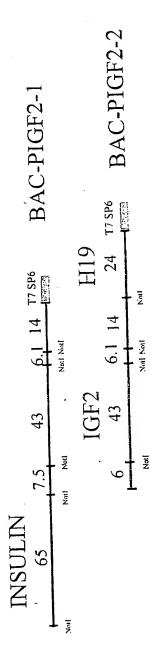
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FIGURE 4



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FIGURE 5



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FIGURE 6

Contig 2 (943 bp)

ACCETATTCCCGCGTGGAGTACACAGCCGAGGAGATTGCCACCTGGTGAGGCCCTGTGACAGCGCTGGGAGGGGCGGGGAGGGGAAGGCCTCAGAATTCCCGCGTGGAACGTGGTGGCCTCTATCATGA

Contig 3 (1500 bp)

GGGGAGGGGATCTCAGAACCCGCTCTGGGAAGAAGAAGACCTCAGAAGAAATCCCTTCCCAAGGGTCAAGGGG
TGGAGCCCAGGGGCCCGCTAGGGGCCGGATTCCCAAGACTCGTGCTGCACCTGCTGGCGCTCCCAGGAACTGC
GGAGCCGGTGGGGCCCCTGGATGGGTCCGCAGTGGGCTCCCAGGAGACCCCTGCAGGGGCTCCCGGACCCCC
AGCTGCCACTCACAAGGTGCCCAAGCGGCGTGGCAATCGGCTGAGCCTCTCCCCCCCTCCTCCTCCCCAGGA
CATTGGCCTCGCATCCCTGGGGGTCTCGGACGAGAAATTGAGAAGCTGTCCACGGTGGTTTCTCCCCCTGC

AGGGCCTGGGTTCCAGCCAGGCCCTCCTGTCCAA
GGGGTGTCCTCACGCTGTGACCCCGGGAGCCTGGATCGGTTCTGCCTGGCTTGGCGGGTGCCCGGGCCA
CGGGCAGCAGGCGTGCGGGGCCCAGGCGTTCTGAGCCCCCTTGCCGCCTTGCCCACCAGCTGTAC
TGGTTCACGGTGAGTTTGGGCTCTGCAAACAGAACGCGAGGTGAAGGCCTACGGGCTGGGCTGCTCCT
CCTACGGGGAGCTCCTGGTGAGGCCTCCCCACGCGCTGGGCCTGGGTCCCCGGGGGAGGTGAACCCCTGCGG
TGCCTTGTGGATTCCAGCTCTCGGGAGCTTGAGCGCTGCGCCTCCTGGGGCACCAAGAAAGCTGGTC

AGGCGGCTCCAGGGAGGAATCTTACGGAGTCAAGGCCCGGGTGCCGCTGGTCTCCGAGTGACATUCCCGTGGT GTCCCRTCTGCCGGCCCACATGCCCCTGAGAGAWGCCCCATCCCCTGGCACGGGGCCCCGTGCCGGGCAGGC GGCGGGAGGCCCAGGACCGGTGGCTGCTGCGGCTTCCACTCCAGCGTGGCGGGGTGGGGGTGGCTGTCTCT GTGTGACCGGCTCTCCCCCCAGCAGGTGCCGTGGAGCTGGGCGGAGGCCTGGGGGGCCTGCAGGCCCTGGCGC TGGAGGGGCCCCCGCAGAAGCGTGGCATCGTGGAGCAGTGCTGCACCAGCATCTGTTCCCTCTACCAGCTGGA GAACTACTGCAACTAGGCCGCCCTGAGGGCGCCTGCTGCTCCCGCACCCCAAAACCCAATAAAGTCCTGAA GGGGGGCTGCCTGCGACCCTCTCTGCTCTCGCCACATCGGCTGCTCTAAGCTTCCTCCACATGCATCGGGT GCCCACAGGCACATGGCCACCGGGGGACCAGGGCCCAGGGCAGGGCCCTTCAATGTGGCGAGCTCTGGTTTTC CCCCGCGGGACCAAGACCTGGCCAGCCTGCCAGTCGCCCAGGCCAAACCAATCTGCACCTTTGCTGAAGGTTC CACCCGGGCCAGCACTGGGGGGGGGCCGGGCCTAGAGCTGGGCGCCCGGGGCCCCAGGGACTGCACACCCGCCAG AGGCTCTCACCTGTGTCVTCXCCTCCCCACGGCCACACAGACACCCCTGGGGAGAAGTCACAGGCCCCCAGCA **GCACCTGCAGCAGCTGAG**GTACGTGGGGTCCCCGGACTGGTTGGTGTCCCGGCTGCCCTCTGGGAGCCAGCGGG CTGAGCTTGTGGTCCTUUCAACCAGGGAGACCCGTGACCACCCTGCTGCTTCCCCTCCCCUCUAGGGCCAGCA GACTCCTTTGGGACTCGGGGCCCCTGAGCCGCCCCCACTCGCAGGACTCACGCGGTGTGCGGTCCTGGGTGAG TGGGGGCTTGGGAGAGGGTCACTCTTGTCCGTCGGGTGGGGAAGGCTGAGAGTCATGGTGTGACAUCUCCCTC GGCCTGCCGGGTGGGGGGTCTCCCCTTCTCCCGAGCCCAGATCCCCGGGTAC

ACCAGCCAAGGTGGTCCGAGCGGTCATTCACAGACAGAACCAGCAGAGGGCGCCAAAGCCCCACTTTTGACAA
ACTCCCCTTCGCCCTGAGCCGAAAGTCCAGGCGGCAGGTGGACCTCTCTGCAGGGCTTGTCACCCCTGCTGC
CGCTTGCCAGCACTCACAGCGCCTGCGGGGGTGCCCAACAGGCCGGCTACCCTGAGCTCTTGGAAGCGATTGA
GTTTAGCAGGGAACGAGGGGACTCCTGGGGGTGACTTTCTCAGCGCCCACATTGCGGCCCAGCAAACCGAGG
CTGGAGGAGGCCGCGCCCCTGTGUCCAGCTGGAGCCTTTCCTCAGGGCTTCCAAAGCCTGGGGAAATTGAGGC
TGGGGGCTGGGGGTCTCACTGTCCGGCCAGGAGG
TGGGGGCTGGGGGTTCTCACTGTCGGGCCAGGAGG

Contig 6 (4833 bp)

ATGTEAGCTCCACAGCATGAGCCCTCGGCCCACTGCTGTGGCCTTGCGGACATTGAGGTGTGTGCCGCCAG
GCCGACCACACCCTGGCCTCTCAGGGTGCCCGTACAGAGCGGCTGGGTCGTANGAGGTGCGGGGCTCTGGGG
ACCGCTGGTGAGTTCAGGACGGGGGTCATGCCACTTCTTCTGAAGGTTTGGTGAGGTGGCCCTTCTCTTAT
CGTGATGACAATACTGATTTCTGGAAGAGCCAGGTGTTTTCTGAGGCTGTGGTCACTTCTCCCACGTGGCCA
CAAGGTGCCGGGCTCAGATTTGAGAAGCCCTGCGGGAGCGGGTGTCATGCGCCAGATTCAGCTTGCCC

CCGCTAGAACACAGTTTCCTCTGATTCTCAGAAACCAGCAGATGCTTTAGGAGGGGCGTGCAGGTTTCACCTG TGCTGCANNGCCCCCTGCCACCTGGTCGGAGCCNCAAGACGGCATCTAAAGATCAGTTCCTCATCATCAGTTC CGCAGTGCTGGGGTGGGGCAGATGAGAACUTCAGGGCTGGGCGCAGAGGTGGGGAGCCCGCCTGGACCCCGA CACTGCAGGGGGGCCTCCCCCTTGTAGGAAGAACAATGTCGCTTTGCCACCCAGCCCTCTCCCCAGGGTGCCC CGAACTGTTGCTCCTAAGACCTCTGGGCTGTGTGTGTTAATTCTATAAGTGGCCACCAGGTGTCAGCAGGAGG CCACTTAAGCATCCATGTGGCGGAAACCTGGAGCTGGGGGTTCCTAAGGGTCCCTCGAGTGTCTCCTGAATAA GTGCGAGGCTGCGGGTCACAGCCCTCACAGCCCCAAAGCTGCAGGTCCTGCCTCAGGGGCACCGCAGCTTGGC TGGTCCCCCTTGGGTCCTCCCCACCCTGACCCGTCCTCTGCTCCCCTTTGCTTAAATGCTCTGCGTTTC AAGGTTCTGATGGAATAAAATAGCCCTGCACTGGTGTGTCCTCTTTGGGGCTGTGCCAGAAGTGGGAATTCA GACCAGGGCAGAGCTCAGATTCCACATACTGTGTTAGGGATGGCAGGTGCCACATTTCCAGGAGTTTCATTGG TGGTTTGTAAATGCTACTTCCGTTTCAGCCCCTCAGCTGCCCACCTCCAATTTAGGGACCCCCCCTTTGG CGGGTTGCCCATGGAACCACATCATCTGGCGTGGGGTGAGCCCTTTATCCTCCCTGGCCCCACTGGGAGGGTT TGGGGAAGTCCCAGCTAAATTTCTCCGTAGGGACCTGGAAGGAGCCCTTGTGACATCTGGGCACAGATAAGAG GTAGGGGGCACAGGCCGTGAACACTTGAAGCTGCAGAGCCCACAGCAGGAGCCAGGAGCAAGTGACTGCTC CCCACCCAAGAACTGTGGGCTGCGTCACACACTCCCCACTGTGTGCCCTGGACCTGACAGGGCCTTTAGCCT  $\verb|cctgcatcccccacccaagaacccagtgaagicaccccacttgcccctcttaatgtttattggctctg|\\$ GGGCATCTGCATTTTGTTTAGGACACCCCCAGCTAGATTTAAGTCCCCCCAAGTGTGACTCTTTCCTCCACTG AAAACCCTGTCCTCCCACCAAAGGGCCCTATCCCTTTAGCTGAGCCAAGGAAATTCAGGAGGGCCTTGAATG GGGGTGCAGTGAAGGTAGCCGCTCGTGGCCTTCTGGAAACTACATGTGACTTTCCCATTAGGTGAGTCTTTGC TTTGCCCCTGCTCTATCTGCAGGCTTATGCAAGAAGTTTAAATTCCCAGGGACACTTGGTCTAACCAGGCAGC GCTTGTATCTGGGCCCTTCCCCAGCTGCTGACCACTCTGAGTCTGCGCCTTAGTTGGAGTTTTGGCCAAGCTC AGTATTGTCACTGTCCGGCACCACAUAUATGGTGCAGGGGGTGGTATCAGGTGCCACTGGGGAAGGGAGAAAA CTCCCAGGTGAGTCCCCTGCCTCTGGAAGCAAGATGCACATGACCGCACTGTGTTGCAGCTGCATTGGGAGGC CCCGAAGAAGATTTTCTGGATCTTTCTCGAACCCTGCTTTTCCCCATCATGCCCCGCCCCCCATTTTACCCGT GCCACGCCACTGGTGTGCCGGGGTGTCAAGTGACTGACAAGTGTCAATCTACTGAGGCCCTGCCCACTCTCC ACCCCCCACATAGTCCCACCTCCCAGCTGGCAGGGAGAACTTCCAGCTAATGCCCATGCCCACAAATGTCTT TCTGTCAGCCTAGAGCTGGACCAAATCTCCACCCTGTAACATGCTGTGCCCTGGCGTGGGAAGGTGCCAGAGC CAGTTGCCCCAGCAGCCCCAGAACCACTAAGTTGGCACACAAGCTACCCAAATTTGGAGGGGCTTGGGGAAGGG CATGGAGGGGATGAGGAGGTGAGGGGCAAAACTAATTTCACTTAGCATTTGAGCAGGTGCCACGCTCAGCGTG GAGAGGETCTCTTGCTTCTAGGGACCCATTATGATGCACGCTAAAAGCGCCCTTCACCATCTCTCCAGCCT CAGCTTTGTCCCCCTCCTCCTCCTCAGCGGCAACCCGGCTGGAGGGTCTGGCCACTACAGCCAGAGCGCCCCC TACTTTGGTGGCGACTGCTACTATTGGCCCAACCAGCGGATCACCGGCCAGGCAGTTTCGGCAGAGAGTCTGG GGCACCAGTGACTCCCCCCTCTTTTATCCACCACCAGGAGCTTCAGGGACTACACAGCGACTAGAGGGCA GGTAACTGGTCTGCCCTCCCTAGGGCTGCCCCCTCAGAGTGTGTGAGAAAAGCTGCATTGAGTGTTTGGGTGC AGGTGGGCTGGGGGCTTGGGGCAGCCAACAGGAACGGCUUGACCTCTGCTTCCAGAGGACCCCAGATCCTGCC AAGCTTCGACTTTGGAGGGGACAGGAAAGACAGGTGGAĞAGGGGACACTTCCCCTCTCTGTACAGACGCCCAC CCGGAGCCACAGAGGCTTTTGCAAGGAAAATAGGTTTTCCCTCACTAATGCAGCAGGCAAAATGGGAAGGCCA GGGGTGGAGGGTAGTGCCCCCCCCCCCCCCGCAGGAGGGCACAGCTGTTTCTGCAAATGTAAAAAAGCAGGGTTT GGGGCCAGAGCCCCGAGATTTTGGAGTTGT?"ITTATATGCATATATACCATTTTGAAAGCAAAGCTTCCCTCT CCCCTACTCCCTACATGTCCCCCTTCACCAAAAAATCCCACCACCTAACTGGAAAGGGGGGGTGAGAAGGACGA CGAAGGGGCACTGTCCCCTCCCGTCCCACAGCGGGACTTAAAACGTACAGCTTTTCSCCTCCGGACAGTGTGC CGCCCCTGGCCCCGTCACGCTCCCCTGCCCGGGGGCTGAGTGTGGGGCCAGGGCCTGTCTCCAGGCATGC ATTATTTTGTGCATGAAGGTTTTGTCCCGCCCACCCAGGCTGGTGTTGGGGGGAAGGGGTTCATTGCTCCAAA GAAGCCCATCTCCCCCCTCAGCCACCTTCAGCCGCCTTCGCAAGGCAGAGCTGTGTCCTCTGCTGTGTGCCTG CCCCGAGGCAGGGCATTTGTGTGCGGCCCCCAGCCCCAGGCCGAGGCAGATGGGCCAGCCTGCCCGACAGA ACACTCTTTATTTTCCCCAGGGGCCGAAGAGTCACCCCTGAACTTGAGGACGAGCAGCCGGATTCCAGCCCCC AGCCCAGGGCCCAAATCTCCTCGGGCTCAGCCGCGCGCCCAAGCTGCCCCCAGCCTGAGCTGCAGCAGGC CAGGGCTGCCCGAGACCCCCAGGCCGCCGGGTGAGCTGCTGCAGCCTGTGGCCCAGGAGATCTCCGCCGGCTCAG AACTGAGGCGGGCAGCCCACCCAGCCGCTGAGTGTCTCCCAGACCCCAGGGCAGGGCCCGGTGTCCCC CGGCACAGAGAGCTGTGCTGCAGGCCCAGACCTCCCAGGCCGTTTTAGTTCCCATCTCCCCTTGGGGGAGGGG TGGGGCTCAGAGGGGCTGGGGTGCATCCGCAGAGCTGGGGTGCAGGGCTCCAGGTGCCTCTCTCCCAGGCGGC TGGCCCGGAGGGGG Contig 7 (2014 bp)

PCT/EP99/10209 WO 00/36143 11/48

FIGURE 6, CONTD.

GCCGCCGGGGGTGCTCCCGGGCCCCCGGACCGAGCCAGGGACGAGCCTGCCCGCGGCGGCAGCCGGGCCGCGG CGGCCGACCGGGGAGCCTGGGGGACCCCGAGCGCCCGGGGAGCAGCGCCCCGACACGCCCCGGGCCGCTCTCG GCTTCCTCCCTTCCAGCCGGCCGGCCGGGCCGGGCCTTCGGCACCGGGGCGCTCTCAGTGGCAGGAGAAGCG TGCGCTCCCGCGGGGTGGGGGACCCGCAGGAAACC

CGTCCCCGACGCCCCGCGCGTTCCCCGGCCGGCATCCTAACCTCTCTCGGTCGCAGCCCCGCAT CCCCAGGGCTCCAGGCCCCCGGCGACTTGCCCGCTCCTCCCAATTGCAGACACGACTTTTTCTGGGACCTCCC  ${\tt AAAGGACAGCCTGGCTCCAGGGTCCCCCAGATACATTCACCATTTCTCCAGATCACAAGTGGGTTTTTCGGGC}$ ACTAACTTCCAGAGACCTCAAAGCACATGAGCCCCTACTGGCTTTCCCAGGTTTCCACTAGTGGCCTCGGTCC CCACCTCACTGGGGATTGTCTCCCAGGCTCTTCGC

GGTGTGATCCCACCCATTCGCGCCCAGGTCCCGCAGTGCCAATCCCTCCTCTAGAAAACTTAAACACTGACTC  $\tt CTGGTCTCGGGGTGAGGCTGCCCAATGTGCCTGACTCCCCAGAAGGTATACCAGTGTTTTCTGGCATTTGGG$ CACCGTTCCCCCAAAACACGTGAAGCTCTTTTCCCGCGTCCCCATAATTTTGGACGCCAGGGGCACCCAAGCT TAGCGCCCTGTTTGGCTCCCCCACACCGCGAAGCCCTGCTCCCTGGGGTTCACGACAGTTTGGGACTTTATC CCCAACTCCAGTACAGGAAGCGATGGCCCCAGGGA

CCCTCGGAGTTGGAACGTGGCTTCCTAAGCCTTCACCAAAATTGAGGCTTTCCGCGCATGGCGCGTGATGCC CAAACTCACTGAGCAAAAATCTTTTTGTGGGGGCTGCAAAGATAGGAGGCATTTCTCCCCGAGCTCTCCCAAA CTCCCTTGCCTATAATCAAGTTCCCTAAAACTTAGACAGAGCTTCCCAGGCCCCAGAGGCACACAGAGCCATT
ATTGGAGCTGCGTTTAATGATGACAGGGACCATGGGTCAGCAGCTCCCCCAAGTCACAAATGCCCCAGGTAT CCTTCGCTCCAGCCAAGCCAAAGCAAACTCTTGC

ACAGATCCCATATCTTGTTATGTCAAGCGCTTTCCGTGTCCCAGTAAACAAATAGTCTGAGTGTTTTCTCCAC CTCATAACATTCGGAATATTAAAAAATTCCCTGGCCCCCGGAGCTGACAGAACAAGAATCCGGGCTTCCTAAA ATTCAGAACTGATTCCCAAATCCCCAGGCCAACGCCAGACCCTCTCCCAATCTGGAGCCCCTCCGACTGGACAC ACTGGACTCCTAAGTATTACGCGCTGTCCTCCAGGCACCCCAAATGCATTCAAAGTGACGCTTTGGTCACAGA AAGGCACTGATTTCTTGGGCTCCAAAGCAGCCCATGCACCCCCGAGTCACCCCAAACTTAGTCAGCATTTCCC GGGTCTCCCTCCGCACTGCAAACTCCCAACTGCGG

ACACCGGTTCTTCAGGACCCACCGCCTAGACGGTCTTAATCCCTTTTCCCCCAGACCTAGATTC

Contig 8' (371 bp)

CCATTGATTTATGGGTCCCCTGACTTTATGACCCTTGCCCCAAGTCCCCCTAAATGTAGGCCATTTTECACGG GCCTCCCAAAATGAAATTGCCCAGATCCCGCGAAAAAAATATCCCCGGGTCCTGGAAATCCCAGGTATTACA GGCCTGCGGCTGACACCCCTCCTTCCTACTAACCAGGTTCCCTGAAGTTTAGAGATCACTACCTAATGAACAA **ATCCAC** 

CCAAAACTGGGGCCCTATCTTACTAGGGTTCCCTAAATGCAGACAGCGCCCGGGAAAATAGGGGGCGTTTTTTT TCCTGTTTGCCAAAAATAAACTAATTGAAACCAATTTTTAGAATTAAAATCTAAAATGACCTTGATTTTCTGC GTTCTCCAAATGTACTTTTCACAGCCCAGGTTGCCCCCCAGTTTAGACGGTGTTGCTTGAATCTCTAAAGCACC CCCAAACGTGCCCGCTGTTCTTCTCCCCCCAAATT

TTATTTAGAGAATATGCCTCTCTCGGGTTCTGCCAAGTTCCCCGCTGAGACTTCCTCGGTCATCCCCAAATCC TCTTCCCCACAGTCCGGGAGCCCCCACAAGCTTACCGACCCACATGCTGGGGTCCCCCAACTTAAACGCGATCCCCTGTCCCCCAGATTCACCGAGTGATTTCCCTGGTCCTCAGACTGGGACTCTTTTACTGGAGTCTCGAATTT AGCCATTAATCACAGTTCTCCACTCCGACGCAGGCTCCCTTGGGTCCCCACGTCGGGGACATGGGTTCTCTTG CCTGCAAATCAGGCTGCTCTGACTTGCATTCAGGCCTTTGGGCATTGTTCCCCGCCGCCGCGGGTCTCGGTTC TCCCCCCATCCCGCGCACGACGGCACTGGGTCTG

GGCCTCTTGGTGTCTCCTACAAGTCCCCGGAGCTCCTCGGACTTGGGAACTGTCTCTTCCCGTACAATAC ACTCGGCCCGGCAGTGTGTCCGCCAGGACGTAGGCAGAGCTTCTCCCGCGTCCAGGAAAACGACTGGGCATTG CCCCAGTTTCCCCCAAATTTGGGCATTGTCCCTGGGTCTTCCAACGGACTGGGCGTTGCCCCCGGACACTGC GGCTCCCAGCGCGCTTGGGGACGCAGCCTCCGGGCCTCCAGCCTTGCGGTGAGCTCCCCGTCGCCTCGCGTGT CCCGGCCGGCTCCCAAACCCACTCGCCGCCGTCC

CGCTGGGGCTGGCACTGGCCTCCGGCGACTGCCGGGGACACGGGAGCGCGGGGGGGCCTGCTGCAGGCCA GCCCGTCGGCCGGGCCGCGCCCTGAAACGCGCGCGCTTTCGTTTGCTCTTTGCAAAGGTCACAACCGTGG GGAAAACGCCTCGGCGGCCCCCAAGCGGGGCAGGCAGGCGTTGGGAAGGAGGACACGCGGGAGAGGAGCAC GCGAAGTGATTGATGGCGGAGCGAGGGGGCCAGCGGATCGCGGGCTTCCGCCGGCGGCGGCGCCCCTTCCCCTCG GAGGGACTCGGGCGGCCCGGGTTTCTGGGGGCGGG

GGCGCTGCGCCTGCTCGGGCAGGTGGAGGCTTCACGCCGGGCCCGCGCCCAGGGACGACCCCTTACCCCGCAG GTCCCAGCGGGACTCGGGGCCCCCGGGATCCAGCGTCTAGCCACCTGTGCCCGCACCGCGCGGGGGCTTGTGA CACCTACCACCCTGGCCGCCCCCGCGCCCCCGGGACCTAAGAC GGGGCCCCCATACACTTTCGTACACGGATTCGGGATTTCTCTCGAACTCTGCAGATCTTATGGCAAAGTTGA TGGCCTGCATTATTTTTTGATAAATTCAGCGAAAGATGGCGACCAGAGCTATGCGCGTCTGGGTTTTAAAGGC GAAACCCAAATTAACGATCTGGTCAACGAACAGAT

ACAGCATACGTTTTT

Contig 10 (3753 bp)

ACATTCCAATGGGGATCCCGATGAGGAAGCCGCTGCTCGTGCTCGTCTTCTTGGCCTTGGCCTCGTGCTG CTATGCTGCTTACCGCCCCAGTGAGACTCTGTGCGGCGGGGGGCTGGTGGACACCCTCCAGTTTGTCTGCGGG GAGGACCTCTCCTCCGAGGGTCTGAGACTTCAGAGCGGGGGCGCCCTGGCCCTGCGCAGTGATTGGCACCTGC CATGTGCCTGGCTGGGCCTUACACCCCCTGACGTTCCTGCAGCGTGACTCGAAACGGCAAACCGAAGGGACGG GTOCCACGGGGTGGGGAGGCAGACGTGAGTGGCAGGCCTGCGAGGGGTTCTTTCGGGGGGGTGGCCCAGGC AGGCCCACAGGATGACAGCCTGTCCCCTCCTGCTCCTTGACCTGCCCACAGCCAGGGCTGCAGGCACTG ACATTCACCCATGGTATTGTGGTGCCTGACGTCTTGGCAGTGGGCATGGGTTCATGGACTGTTGGATTGAAAG TGGAATAAGATGGGTTGAAAACCAATAAGAATAAAGGCCCUTGTGGCTGGCGGCATCTGCGAGAGGTGACCCC TGCCCTCCCTGUGGTTGGGCTTTGGGTGGGTTCCCATGGGTGGGCUGGCCGCCATGCAGGGTGCCCGCCTGC CACCTCCCAGCAGGCTGGGCCTCAGTCTCCTTACCTGTAGGATCCCTCAGGGGGCGTCCTGGAGAGAGTCCTCG GGACAATGGGGAGGCTGGGGGCAGGCCCAGCCTGACCCTGAAGGTGGGAGTGTGTGCTCCCCCTGGGCTCAGC CCCTGGCCTCTGAGCCCTCTCTCTCCTCCCCCCTTTGGGGGCAGGGAGTGGCACCATAGAATCTGGGGCTGGG CCTGGGGAGCGCCCCCTCGTGCCAGGCTTCCCCGAAAGGAGGGCTGGGCTGAGCTCCCGACCCTCTGGACCC CCTCACTCCTCCTCCCGTCTTCCTCCTCCTCCTCCACTCCCACCTGTGTCTCCGGGGTCCCGGGGCCGCAG GCTGCCCAGGCGCCTGCTGATCCATTGGGGACCGCACTCGGGTCCCCGCTTGGCCTTCGGGTCAGGGCCACGGC CCAAGAGGCCAGGGGTCCCGAGCAGCAGCCGGCTCACCCCGGTTCCCCTCTCCCTTCCCGGGG AAGAGTGCTGCTAGCTGCGACCTGGCCCTGCTGGAGACCTACTGCGCCACCCCCGCCAAGTCCGAGAG GGCTGTCTCCTCTGAGCCGGGGACCGGGGCCCAGCCGGCTCTTGGGCTTCAAGTGCTGCCAGAGGGGCCTTC CAGAGGTTGTTGTGGGACAGGGGTGGGGGGCCAGGCCCCCCCTGACGGCCCTTCCCCTCTCAGGACA ACTTCCCCAGATACCCCGTGGGCAAGTTCTTCCGCTATGACACCTGGAAGCAGTCCGCCCAACGCCTGCGCAG GGGCCTGCCGGCCCTCCTGCGCCCCCCGGGGTCGCACGCTCGCCAAGGAGCTGGAGGCGGTCAGAGAGGCC AAGCGTCACCGACCCCTGACCGCCCGTCCCACCCGAGACCCCGCCGCCGCCGCGGGGGGCGCCTCTCCCGAGGCGT CCGGCCATCGGAAGTGAGCCAAATTGTCGTAATTCTGCGGTGCCACCATCCACCTCGTGACCTCCTCTCGACC GGGACCGCTTCCATCAGGTCCCCCTTCTGAGATCTCTGTACCCTTCTGTCGGGGCATCTCCGCCCGGGCCCCGGGCCCCGGGCCCCCAACCTCCCATGTCAGGCTAGTCTCTCGGCCCCTTCCATCGGCCCGAGGGCATCCAAACCA CAAACCCAATTGGCTTGGTCTGTATCTCCCCCAAATTATGCCCCCAATTATCCCCAAGTTACATACCAAAAA TTGAACCCCTCAACCACACCACATACAATCAGCCCCCGTAAAACGAATTGGCATCTTTAAAACACCAGAAAA AATTGGCTGTGACCCATCATCCAAGAGAAAGGAAGGGACCAAAATTTGCAGGTAGGCTTGTCGCCGCTCACAG CCATCTCCCTCCTGCCACACCCTGGCGGCCACTGGCGGTGTGGCACCAAGGACCCAGTCCCGTCCTCTC TCTAGTCCCATGACCGAGACCGCGGTGGAGTTGGCTGGGAGACCCCGTGAGATCAGAGGAGGGGAGCACGGAA CCAGAAACCCAAACCTGCACAGGTACAACATGACTGGCCCCCGCACAGCCCAAGACCTCTCATCTCAGTCTC 

CACACTCTCTCTCTCTGTGGGATCCCTGAG Contig 19 (500 bp)

TGGCTCTGGCATAGGCTGGCAGCTCTGACTGGACCCCTTGCCTG AAAAAAAAAACAACCAAACAAACAAACAAAAGCCAAAACACACAGAACTC ACAGACACAAGAAGACTGGTGGTTGCCAAAGGTGGGGTCGAGGGTGGG AAAAATGAGGAGAGGGGGCAAAACACACAAACGTGCAGCCATAAAATGGT AAAGTCCCGGGGACCTCCGGTAGCGCGTGTGGGGACTCGGGTTGAGAACA CACCGTGATGTGTATTCGCGAGTTGCTAAGAGTCCCTGTTGGAGAAACAA ATGCGTATCGACGTGTGGAAATGAAAGTTAACCCGACCTGCTGTCGTGAT CACTTTGCAACACATACAGACATAGAATCATTATGTTTTACCCCTGGAGC TGACAGCGTTATACGTCCCCCAGCCTCAATTTAAAAACAGCGTTGCCGTG Contig 20 (400 bp)

TTCATACTGTGCAATGCCAGCCTTAAATGCACAGAGGAGGAGCATTAACTT CTTTCCAGAATCACTGAAATGATACCACTCATGTTTTGCAACTTGCACTT GGGCGTTATTTTATTGGTGCCGGAACAGCGCCGATGTGGCACCAAACTAG CGCCGCTGTTTTATTTCCCCTCGGTATCCGCGCTCTCGCTGTCTTCCCC CCCTTCCGCTTGCAGCTGAGGAAAGGGCTGAGAGGGAGAAGTCTGCATT CACCCATCTCCCCCTGCCTCTGTTGTCATCCTTCACAGAAGTGGTGGCCT GTGCGGGGAAGTCACTAAACCTAGGCAGGTGTCCCGTGGGGTCATGCTTG TTACACCTTTGTGCACCTGGCCCAAGTTCTCGGTGGAGCGAGAACGTGGC Contig 21 (559 bp)

AGCTAGCCCCCCCAGCCAGGCCAGGCCTCTCCTGCCACCCGCCCAGCCA GCATGTCTCAAGAGGAGGGGGCCTCTAAGGGATGAGGACCTGCTCCACTC GGAGACACGAAGCCCCGCCGGCTCCTCCCCGAAAGTCCAGCTGCGGCTTT CGAGCACGGCTGCGCCCTTCGTCAATCATTTCAGCCACAGAAGTGAAAGG CGCTTTCGTGGCCGAGGCAGGCGGGCACACAGAATGGAATCCCACCCCAGA GCGAAGAGCCGCCGTGGGTGAAGCGCGTCTCTGGTGGGGACCGGGCCGGG **AACTTCACATGGGGGTCGCTGTCCCCATCTCCCCATCGTCATTACTGCAG** GGGCTCGGCCACACCCGGAGCTGCGGGGGCCCAGTGCTGGACACTGGACCT GGCCTCCGTCCTATGATGTCATGGCGGCGGGGGCCAGCACAGGGCAGTGGC CACACCTCGGGCCTCCCAGCACCAGCCAGGATGGCAGAGGGCCCCACCCC ACCACGGGGCATGTACATCCCAGAGGACCAGCTGAGCAAGGCTTGATANG GGCTTCAAC

Contig 22 (450 bp)

CATTATCTTCAGCCCCACCGCGCGCGCGTGTTAATGGGTAAACTGGGGCAA GGGGGCCCTGCCTGAGGCCGGGGTGGGGAGCGCAAGGCATGGCCTGTGT GCCCAGCCCAGTCCTTCAGGGCGCTGCTGTCCTGCACCGGGCGCCCCCAG GAAGCAGAGCACCCAGCTTCTCCCCTATTCTAGAACCAGCCCCCAGAACC CTGGACCCAGACCCAGGCCCAGGGGATACTGACAGAGCCACGGCAAGGCG GCCACTCCACACCCCACAGAGGGGCCAGCAAACCCCAGTCACTGCGCAGC CCATGCCCAGGGGCAGATGGGACACGAGAGCAGCCCTCATCCACAGCAG GCAGGGGAGTGAACTGGTGCAAAACGGGGCGGTTCCACGAAAGTTAAGCA Contig 23 (535 bp)

TGCCAGAGACCTCAGAGCTGGGCTCTGCCTTCCCGGGCTGACACGGAGGG GTGTCCCCAGAGGTGGCCCTGCCTCCACGCCCAACATCAGGCCTGCTGCA GCCCTGGACGGCCCCCTGTCCCCCGGAAGCCCTCGGGGCTCTCTCGCGTC GCCTCTGGGGAACCCTCGGTAATGTGGCCCAGCCGTGCAGTGGCCGGATC ATTTGCTCAGGGGGCCCAAGGCAGGGGGGGTGACACATCCGCAAGTACCG CATATGCACAGGATATGGATTGGGTGTGGATTTAACCTTTTCGCAAATGT CTCTGCCGGTACAAATATTGTTTCTAATCCTCTGCCTCCCTGAGCCGGTG AGTCTGCCCGGGGGGGGGGCTGGCTTGCTGAACCTGCCCTGGCCC CCACCCCAAGGGAGCCCCCGGCCAGTGCTGAGGGCAGGAAGCTTGGGCA CAGGCTGCAGAGGCCAGCGCTGGCCTCAGTCACCT

Contig 24 (868 bp)

TATTGAAGACCCTATCATGAGTTCCCAGAGCGGAGGGGTGGAAGCAGGGG CCTACAGCCCACTCCCCATCACTCCAGACCCGTCCGGGGCTGGTGTCCCC TGCCCCCTACTCCTGTCTCTGGTGGGCGGACGCTCGAAGGAGGCACTCTG GCCTGGAGCCTGGAGGTCCCTGAACTCCCGCTGCCACCTGGGCCCTCGG GCTCCTCCTGCGCTGGGACCCGCGGTGGTGGGAAGCAGCCCTGCTCAGTG GGAGGAGGCAGGCTGTGGCCGCCCCGCACGGCCCTGGGGGGGACGCACG

Contig 25 (500 bp)

ACCAGTTTCGATGAGCAATCCCACCGGCGTAACATTATGGCTGCAGCCTG
GTCAATGCCGGTGGAGTTTGAACCTCCACGCGTGGCGATTCTGGTAGATA
AATCGACATGGACCAGGGAGTTGATGAACATAACGGTAAATTTGGCATC
GTTATCCCGGGCGTTGCAGCAACTAACTGGACGTGGGCGGTGGGAAGTGT
GTCGGGGCCGGATGAAGAAAATTTAATTGCTATGGCATTCCGGTTGTGA
GAGGCCCGGTATTTGGTTTGCCTCTGGTCGAGGAAAAATGTCTGGCGTG
ATGGAGTGTCGATTGCTACCTGCGACTTCTCGCCAAGAAGAATACGACAC
GCTGTTTTGGCGAGTATCAGCAGCGGCAGACGCACGGGTATTTCTCG
AAGGCCGCTGGCAGTTTGATGATGATAAGCTCAATACGTTGCATCATTA
GGTGCTGGGACGTTTGTTACCACCGCCCCAAGCCGTTACGGCGGTTAAGC
Contig 26 (900 bp)

ATGTTTGATCTCCGCGCGTGCTGTAANATTTACGCTGCTCGCGTTCTTT GGCTTCGTCCACCACCGGAAAACGGACAAAAATTTCCGTCATACCTTTTT CTTTCAGGCGGAAGCCAATGTCGTAATCTTCAGTAAGACTCTGCACGTCG AAAGCAATACCGTCACCGTCAGCTAACAGTGCGGTCACGGCGGCGGCG GAAACAGGTGCCGACGCCTGCGCTGGGCACTTGTCCGGCGAGGGCTTCAC GCACCGGAACATCTTTGCCATGCAGCTCTGAAAACTCATCAATGTAAGTC ATGCTGGTGAAGTGCGTCCATTCGCGTTCGAACGGATACACCGGGATCTG AATCAGATCTTTACGCTCGACCAGATAGTTGAACAGACGCAATTCCATCG GTGAAATCACATCTTCGGCGTCATGCAGAATAAAACCAGCAAAAGCGAAA TTGGCGCTACGCTCAAATTGGGTGATGGCGTCCAGCACGTTGTTCAGACA GTCGGCTTTGCTGGTGGGGCCAGGACGCGCGCAGACTACCTTATGCACAT TCGGGAAGCGAGCGCACACTTCGTCAACATCACGCTGAGTATCGGGGTCG TTGGGGTAGGTGCCAACAAGATATGATAGTTTTCGTAGTCGAGCGTGGT CGCCGCCAGCTCGGCCATATTGCCGATGACGCCCGTTTCATTCCACGCCG GAACCATAATCGCTAACGCTTTTTCATCTGGTTTATACAGTTCGCGGTAA CTCATTCGCGGGTAGCGGCGATAAACACTCAACTTGCGTTTAATGCGGCG TACCCAGTATACGACATCTATAAAAAAATCGTCCAGCCCGCTGATGAACA TGATGACCGCTAACGTTATCGCGATTACTTTTAAGCCGTATAGCCAGGTA Contig 27 (500 bp)

AGTECGGTCGGGCCGTCCTGACGCTCAACACCGTATTTCCACGCGACCGC
GGATTCAACCTGGTCACACGGACGCCATGTAGACATGTTCGGGGTTACGC
GCAGAGAAGCGCCTCCACCGGCTGGTGAGTCGGGCCGTTTCGCCC
AGACCGATGGAGTCGTGGGGTGTAAACCATCACCTGACGCTGTTTCATCAG
CGCAGCCATACGTACGGCGTTACGTGCTTATTCCACGAACATCACGAAGG
TGGAGGTGTACGGCAGGAAGCCACCGTGCAGGAAGATACCGTTAGCAATC
GCGGTCATACCGAACTCGCGAACCACCGTAGTGGATGTAGTTACCCGCAGC
ATCTTCCTTGATTGCTTTAGAACCAGACCACAGGGTCAGGTTAGACGGCG
CCGGGTCAGCAGAACCSCCGAGGAATTCCSGCAACAGCCGGACGAACGCT
Contig 29 (450 bp)

TCAGGCCAATCTGTCTGGTCTCCAATGGGGACAATTTGGTTCTTTAGGCT TCTGTCCAATGGTCCGAATGGCCCACTCCCCGGGCGCCGGCCAAGGGTCC TCTGTGCCTCGGGTGGGCTGGCACGGACCGCCCCCAGGGTCGTGCCAGCC CCGTCACCGGGCCCAGAAGCTTCGGGCCTCTAGCTGGCTAGTCGGGCTG CTGTGCAGGGGGCTGCGCTGGGGGGCAGAGCCGGGGGTGAGGTAAACCTC CCAGCCGCCGGGGTCCCTGCCGCAGCCCTAGGCGCCGAGACGGTGGCTG GGTCGGTACCGCAGACCCGAGGGCCTCGGGGCCCGGGTGACCCCAGCTG TCGCACACGCTCGCAGCTCTCTTGCTCATCAGGGCTCATCCCTCTGGACC TCTCCTACTGCCCCACCTCACCCCGCCTGGACCCCATGAAGCCCCGCGGA

Contig 30 (600 bp)

TAAAACTAGCTCTAGTAGAAACATT"TATTTAAAAATAAAAACCTGACT ACGTCGGGAGTTCCCCTTGTGGCTCAGTGGTTGACGAATCCGATGAGGAA CCATGAGGTTGCGAGTTCGATCCCTGGCTCGCTCCGTGGGTTGAGGATC CGGCGTTGCCGTGCGCTGTGGTGTAGGTTGCAGATGAGGCTCGGATCCTG CGTGGCTGTGGCTCGGGTGTAGGCCGGCGGCTACAGCTCTGATGAGACCC CTAGCCTGGGAACCTCCACATGCCCTGGGAGTGGCCCTAGAAAAAGGGCA AAATGAAATTAACGACTGCCTAGGGTGGGATTTACAGCATGGGAAGTACA GCATGGCCGTGACAGTGCAAGGGTGAGGCGGGAAAATGGAAATAGGTTAG GTGAGTTTCTCCTGCTATTTGTGATGTGGTCTGCTATCGCTTGAAGACGG ACTGCACTGAGATAAATATGTACAGTAAGCATCCGAAAAACCGCCAGAAC GGCAAAACGAATGACTCCAAGTAAGAACCCAAAAGGAAAAGGAAATAAT Contig 31 (450 bp)

GCGCGGGCGTTCCGGCTGGGTATTTAACGTGGTCACCGGTTCGGCGGGC GCGGTCGGTAACGAACTGACCAGTAACCCGCTGGTGCGCAAACTGTCGTT TACCGGTTCGACCGAAATTGGCCGCCAGTTAATGGAACAGTGCGCGAAAG ACATCAAGAAAGTGTCGCTGGAGCTGGGCGGTAACGCGCCGTTTATCGTC ATTCCGCAACGCCGGGCAAACCTGCGTCTGCGCCAACCGCCTGTATGTGC AGGACGGCGTGTATGACCGTTTTGCCGAAAAATTGCAGCAGGCAATGAGC AAACTGCACATCGGCGACGGGCTGGATAACGGCGGTCACCATCGGGCCGCT GATCGATGAAAAATCGGTATCAAAAGTGGAAGAGCATATTGCCGATGCGC Centig 32 (450 bp)

GGTGGATGCTGGCGATAGCGTCATCCTCGCTTATGCCGTGCAGCGGGCAA GATACGCGCCGCTAAAAGCGATGGTGCCGCTACGTTTGGTGGCGGCGCG GCGATTTTTACCGCGTTTTCCACCGCTTCGGAACCGGTCGTAACCAGCAG CGTTTTCTTGGCGAAATCGCCCGGCACCTTCTGATTCATAATCTCGCACA GCTCCAGATACGGCTCGTAAGCCAGCACCTGGAAGCAGGTGTGCGACAGT TTTTCAACTGCGCTTCCACCGCGGCCACCACCTTCGGATGCAAGTGCCC GGTATTGAGCACCGTAATCCCGCCCGCGAAATCAAGATACTCACGGCCTT Contig 33 (500 bp)

ACGTGAGGTTTGGGGGAGGAAAGCGGGGGACGAGCAGCCCGAGAGGAGTG GGGGCTGGCCTGTGGCTGATGAAACTCTGAGAAGGTTAAGAGCCCCCATT TTTGTCTTCCTCTTTTTTATTATGGAAAATTCCAAATGGATGCAAAAGTC CCAAACCTAACTGGACATCTTCTTGGTACCAGGAACGGTCAGGCACTTAT GATGUACCGAGCCCCGAGGGAAAAACCCTGCCGTCCTGGAGCCCACGGTC CAGCAGGGCACACAGGCCCCAGCCGCAAGCGGCACGGCTGAGTCAGTGA ATGGCGTGCCCTCTGGTCAAGGACGGCACTCTGGACCCCAGGGAAGCCT CTGAGGAGCCCCCTTCACAGCGTCAAAAACTGTTAACAGGGCCATGTTCC CACCCCCCACACACGTGGTTCAGAAGCAGACCCCAGGCATCGTAATATG TCATCCGTGAGTTCCCTGTGTGCCACCAACAGAAAGCCCATCGTCACGTT Contig 34 (400 bp)

CGGCATCGATGTACATGGTACGCAAGGCACTCGTAAGGCCCCGAGCCTCT AGGCCTTGTCATTGTCACGTGCTGCTCGCGGGGATCAGCAGCCAGGCTTG TGACCCCGGCCACTTTGACAGATAAGGACACAGAGAGGCCACAGCACTGG TGTGAGGCCCCACAGCCAGCAGCCCAGGGCAGGAGGACTGGGTCTCACC TGCCTCAGCTGGGCCCAGCCTCCCTGGGAGTCCCCGGAGTCTCCCCAGCTT CTCCCCTCTGAGGACCTCTACTGTTCCTCCTTATCCTCCCCTTTGAGCCA Contig 35 (500 bp)

TGGCGGTGAACTATGTCGTGCGTGAAGAGCATTTGTGGTCGGTAGCGCGT

TATATGCGGGAAGTTTAGGCGAACTGGACAGCCTGGGTTTATCCGGTAGC
GAAATCCGCTTCACGGTAAAACGCTGCTAGCGTTGGTTGAAAAACGCA
CACATTGCCGGAAGATGCCTTACCGCAGCCGATGCTTAACCTGATGGACA
TGCCGGGTTATCGTAAAGCCTTTAAAGCGATTAAGTCGCTGATTACTGAC
GTGAGCGAAACGCATAAGATCAGCGCCGAATTTACTGGCGTCGGCA
AATCAACCAACTGCTGAACTGCACTGGAAACTGAAACCGCAGAACAATT
TGCCGGAGCTGATTTCCGAGCTGGCTGGTTGAGCTGATGGCGGAACCATT
ACACAATTTATTGCAGGAATTCCGCAGTTAAAATCCTCCGAAGCCGGACT
GGGCGCCCTCAGCCCAACATCCGGCTTCGGCAAACTACAAATCCAACACC

GGGGAGATTTGTTCGATGACCGGAAGGGCAAAAATTTTCTTAATCATGAC

GCAGTCCTTTAACTTCATTTTTTCAGGTAAAAAAAAGAGCGACCGAAGTC
Contig 37 (300 bp)

ATCCTTTTGGGGTCTGGCAATTACGCAATAAAGAAGGCCCCCATGCGATT
AAAGTCACCGGCCCACTGTCGTCTAATCATGAGAAATTGCCATCAGTG
GGGTCTCGATGGGCAGGGGATTTGCTCTGCTTCTGGGTTGGATATTACCG
GAAACATTGCCAGTGGTCATTTAGTGCAAGTGCTACCGGAATATTACCAG
CUAGCGAACGTCTGGTCCGTTTATGTTTCAAGGCTGGCGACGTCAGCGAA
AGTGCGGATAACGGTAGAGTTTTTACGCCAGTATTTTGCCGAGCACTACC
GGAATGTTTCACTGTTGCATGCCTGATTTATGATTCAATTATCGGTTGA
TATCAGTTTAAAACCTGATTTTCTCCTTTCTAAGCCGCTACAGATTTTGCT
CGCATATTTCACCTTTTAATCGCGCATGATCTAAAGATAATTGAAGAGGGTTA
CONLIG 39 (450 bp)

AATGTACTGGCAAAAAGCCAATGGCGAAGCGTGGGGAACCTTACATGCTC
TGCTGGCGGATATTAATAGTCAGGGTCAGGTGCAGATGGCGATGAACGCC
GGCATCTATGATGAAAGCTATGCGCCGCTCGGTTTGTACATCGAAAACGG
TCAGCAGAAGGTGGGGTTAAATCTCGCTTCAGGTGAAGGTGAATTTCTTTA
TCCGTCCTGCCGGCGTGTTTTATGTCGCGGGAGATAAAGTCGGCATCGTT
CGTCTGGATGCCTTCAAAACCAGTAAAAGAGTCAGTTTGCGGTGCAGTC
AGGGCCAATGTTGATGGAAAACGGTGTAATTAATCCGGTATTCATCCCA
ACGTCCCTCCAAGCAAAATTCGTAACGGTGGTTGGGATTAATAAACATGC
GAACGCCGTGTTTTTGTTGAGCCAGCAGGCAACAAATTTTTATGATTTTG
CCDLEG 40 (400 bb)

GCAAAATCACGTCCGCGACCTGGCGTTCTCGCTGGGCCATATTGGCAAAG GAGCTGGATTGCGGTGCCTGCAAAGTGCCCTGAATAATGCCATTGTCCTG TACCGGGAAGAACCTTTCGGAATGAACACCCAACGCAACGCATAAGCA GCAGCGTGCTGAGTGCCACGCTTAAGGCTCAGCCACGGAATGATTCAGCACT TTCGCCAGTCCACGACCATAGGCGGCGATTATCCTGTCGAACATTTTTC CGAGGCACGGGAGAAGCGGTTCTGTTTACGCAACGACTCCTGGCTGAGCA TCCGCGCGCACATCATCGGTGTCAGGGTCAGCGACACCGCTGAGAT

Contig 45 (300 bp)

AAAATCGCTACCGCCAGGGTAATAGCAAATTCGCGGAACAGTCGCCCGAC GATATCGCCCATAAACAGCAGTGGGATCAACACCGCAATCAGTGAGAAGG TCAGCGAGATAATGGTAAAGCCGATTTCACCTGCGCCCTTGAGCGCCCCC Contig 42 (400 bp)

Contig 44 (750 bp) GGTGCACTGAGTCACAGGATGGCGGCGGTGGTGGGAAGCGGT CCTGGAGGGCTCGGGAGGAGGATGCGCTCAAGCTGGCTCCCCGTGGGGC TGGCCCGGAGTAGCCTCCGTGAGGGCACCGTGTCTGCTCCCAGAGCCCGC TCCCCGGCCTGCCTCCCTTCCCTGCCCCAGTTCCCCCGGAGCCCC AGAGCTCTGAGGCCACCAGACCTGGCCAGGACCCTTCGTGGGAAGAAGAG CTTCCAGGCGGGCTTCCAGGCAGGCCAGTGTCCTGGGGGCTGGAGCGA AGCGGGAGGAGACTGGAGCCAACTGGGGGGACAGAGGAGGGGTCCAACCC CAGCGGTGGTGTTGGGGGTGCTGGTGGTGGAGGCCCTGAGAGGCTGTGCT GGGGGCAGAGCGGGTGCTGGGAGGGGAGAAGGGGTCCCCAGGGCTCATG GGCCCTTCGCAGCAGTGGCAGTTGGGGTGGCTGTCTCTAGGGCTGT ACCACGGTGGGTGCCTGGAGAAAGAGGTCCTACCCCTAGTCTTTGCTGCA

TGGGGACCCACTCCAGCCCCACTGAGTGACGCGCCCCCTGTGGTCCCA CCGCCAACCCTGCCTCACACCACAGGGGCTGTGGCCACACCTTGTCCACA GCCTGTCCCTGAGACCACGAGGCCCCCGGGCTCAGCCCCTCCTCACCCCT GGACCGAGGAGAAGCCCCCACCTGGGCTCAGCTCTTGGAGCTAAACTTCC AGGAAGGTTCTGGTGCCCTCGGGTCTTAGAGCATGGTGGGAGGGGATG CTGGTGGGGGCGCAAGCCCTCCCCACATTTCGCACTCGACCCGGTGGNG Contig 45 (300 bp)

CCGGCTAGAAGCCACGAGAGCCCCCAGGCCCGACGACGTCTCTCCTGC
AGGGATTCGGCAGCCCTGGGGCCACAGGGCCTGAGCAGACCTTGGGGTTC
CGGTGTGACTCCAGCCAGGGTCCCTACTGTTAGGCACCAGGGCAGAGTC
AGCCCTGGGACCATGGCCACAGCTGCTCCCGCCTGAGCCGGCCCCCAGC
CCAGGCTGGGCCCCCTCAGTGCACTGTCCCAAGCCAGCTGCTCTCCCAC
CTCCACCTTCTCCATCCAGGTCCTGCCCCACGGCCTTTGCTCAGGCCAG
Contig 47 (500 bp)

GCGATATTTGGGGGCATATTTGGGGGGGAGATCCCCACAAGGCATTTGGG
GTTTGTGTTTGGAATGCCCCCGGCCCGATGGAGGGGCCCGGGAAGAA
TCTAAGCCTTACTTGGGAGGGTTGGGCCCCGGGGCCCGGAAAT
GCCCCCAAGACAGAAGGGTGTACAAAATTTCTCAAAGGGTGACCCTTAAT
GAAACGGGTCCCUUTTGGAAAGAGTCACCAGGGTGGATTGGTGGCACCG
CAGAATTTACGACATTTTGGCTCTCTTCCAATGCCGGACGCCTGGGGAT
AGGCCCCCCGTGGACGGCGGCTCTCGGGTGGACGGCGTCAGGGGT
CGGTGACGCTTCTCTGCCACCTCCTTGGCAACGGTGG
AGCGCCGCGGCGCCTCTCTGACGGCTCCTTGCCAACGTGCT
GGGGACTTCTCCAGGTGCAACGACCTCCAGCTCCTTGCCACCGTGG
CCGAGCCCCTCTCCAGGTGCAACAACAACCTTTGCCAACGGGGTATCT
GGGGAACTTGTCTTGAGAGGGGAAGGCCCGTCAGGGGGGGCCTGGCCC
CCCAGCCCCTGTCCCAGAACAACAACAACCTTTGCGGGGGTCCTTCCCTTGCC
Contig 50 (179 bp)

ATCTTCATATTCATGCAGAAGACACTCTCCTGCCTTTCTATCTTGGGGAA AAGGACGATGTCACTTATGCAATAAAGCCCACTTGCTGGCCGGGGCTTGA CATTATTCCTTCCTGTCTGGCTCTGCACCGTATTGAAACTGAGTTAATGG GCAAATTTGATGAAGGTAAACTGCCCACC

Contig 51 (500 bp)

TGTGTTGCACCTGTTGCTGCCTGTCGACTCTAGAGGATCAATACTCCTTA CATAATTAAGGAGAACAAAATGGAACTTAAAAAATTGATGGGACATATTT CTATTATCCCCGATTACAGACAAGCCTGGAAAATGGAACATAAGTTATCG GATATTCTACTGTTGACTATTTGTGCCGTTATTTCTGGTGCAGAAGGCTG GGAAGATATAGAGGATTTTGGGGAAACACATCCCGATTTTTTGAAGCAAT ATGGTGATTTTGAAAATGGTATTCCTGTTCACGACACCATTGCCAGAGTT GTATCCTGTATCAGTCCTGCAAAATTTCACGAGTGCTTTATTAACTGGAT GCGTGACTGCCATTCTTCAGATGATAAAGACGTCATTGCAATTGATGGAA AAACGCTCCGGCATTCTTATGATAAGAGTCGCCGCAGGGGAGCCATTCAT GTCATTAGTGCGTTCTCAACAATGCACAGTCTGGTCATCGGACAGATCAA GACGGATGAGAAATCTAATGAGATTACAGCTATCCCAGAACTTCTTAACA TGCTGGATATTAAAGGAAAAATCATCACAACTGATGCGATGGCTTGCCAG TGTAAAAGGAAACCAGGGGGGGGCTAAATAAAGCCTTTGAGGAAAAATTTCCGCTGAAAGAATTAAATCCAGCGCATGACAGTTACGCAATGAGTGAA AAGAGTCACGGCAGAAGAAATCCGTCTTCATATTGTTTGCGATGTCCC TGATGAACTTATTGATTTCACGTTTGAATAGAAAGGGCTGAAGAAATTAT Contig 53 (450 bp)

GGTGGTACATGTGGCCGGAGCCCAGGGCACAGGGTGAGGGGAGAAGGGAG CATGCGGGTGCAGACTCGGAGCCCGCGGGTGAGGTGCTGGGTCCTCAGGA CACGCTCTGGGAGTGGAGGACCCCATCCACGCCCTCACCCAGTGTGTGC CCGCCTGCTCCCCCGGAAACCCTCACAGACACGAGGGCACACCCAGCCC Contig 54 (1133 bp)

ATGGCGCTCATTAGAATTCGACCTCGGTACCTTGGGATCTTTTGACCCCT ACCTCACGCCATCTACAACATTTACCTCCGAATGAATGAGAGACACCAAA AGCAAATTCATAGAAGAGAAAAAAAGGTAACCTGGACTTTAAAAATGTAA ACTTCTGCTCTTTAAAAGGCAGTGCTAATGAAGTTCAAATACAAACCACA CACCATANGAAAATACTTGCAAATCTTGTTCTGACAAAGACTAGTGTTCA GAACATACGACGATCAGGGAGAGGAAAACCAGCAATCCTATAAAACTGGA CAAAGAATTGGGGGGAAAAAAACCCACTTGGCCAAGAAGTTGGTAAATA AGGCCATGAAAACATGCTCAACATCATGAGTCATTAGAAAAATGCAAATT AAAATTATAATGAGATACTACTACACAGCTATTTGAATGGATAAAAAATG TTTTAAAAACTGATTATACCCAGGTTTGGCAAGAACATGAGAAACGAGAT AGAGCTGGGCACTTCCCTCAAAAGTTAAACATACATCCAGGACCTCACAC AGGCTTTCCACCACAGGTGTTTATTCCAGAGACATGAAAGCGCTCATCCA CACAAAGACTCGTAAATGAAGGTTTATAGCACCGTTTGTGGCCCGAACTG AGAAAACCCAAATGACCTTTAACCAGAGAATATCTAAACAAAATATCCAT TCACATTAATCACCCATAAGAAGGAACGGGCTATGGGGACGGGAACCGTA TTGAAGAGGGTCAAAATACATACGCAGCATCAAAGAAGCCTGCCCAAAGG ACACACACTGCAGGGTTCCATGGACTGAAACTCGAGAAGGTGAAAACTCG CCAGCAGTGACAGAGCAGCTCCGAGATCAACCTGATGTGGAGGAAAGT GAACCCTCGTGCGTTGTTGGCAGGACTATAAACTGGAGCACCCCCTACGG ACAACAGTAGCCCGGGCTCCTCTCCTCCATCTCCCTGGGGAGCCTGAGCC TTGAGACGCTGGGGCAAGTGCACGGCATGCTGCCTCACGTGGGGCCCCGG TGAAAACACGTGGCAGCTGGGGAAAGAATCGTA

Contig 55 (735 bp)

#### Contig 56 (500 bp)

GACCCCGTGCGTGGGCTCACAGAGTGTGTGTCCCTCTGTGACCGATCGTC GTGTCCCCGAGGCCCGTTCTGTGGCAGCTGCGTTATGACCGACTACCTTC GAATGCTCAGTGACTGCCGTGCATTGGACACGCAGTCCGCTACCCTTTTC Contig 58 (550 bp)

"GAGGAGCGCAGGCCCAGGCCTGAGTGTGCCCAGCTTACACCCCTGGCAC CTTCGTCCCTCCTGGCCCTAACCCCCATCCTACCCCAGCAGCAGGGGGCTC CCCCGGTGGGCCTGCTGAGCGTCTGACTGGGGTTTGGAGTCAGGTCTGC TOCAGGCTCAGCCCCATCCCCAAGGGTGCCCTGCAGCACTGCTGCCCAC CCCCTAGCGCCCCAGACCTTCGCCCCTCCAGCCTGGATGTACCCACGGA CCCTGAAAAGTGGGGCTGAGCAGGTGCCCTGGCTGGAGTCCCCCTGACTT GGGGCTGGCCAGGCTGCCCCA TCCTGGGGGGCCACACCCTGCCCTGGGGTTTGGGGCCAAGCCGGGCACG CCCCATGTCAGGCGGGGGGGGAACCAGGTAATTACAGCCTGGCAGCCCGCT CCCCAGACCCCCAGCCCGGAGGGCCCCCACCCAGGCTGTGCCACCAAGA CCTGGCATCCAGGGCCCAAAGCAGGTCAAGGGCAGCTGCTACAGATTCTT TTAAGTTGAGACAGAATCGACACATGACAAGTTCCTGGTTTTAGGTACTT CGCTGCCGGGGCCGCCAGTCAGTTTAGTGACCCAGCACACCCCACACAGG TACAATTGCTCTTCTCAAAAGAGGCCCCTGAGAGAGCGCCTGTCTTGGCT CAGGGGTAATGAGCCCAATGGGTATCCATGAGGTTGCGGGTTCCATCCCC GGCCTCGCCGCGTTGGTTA

Contig 60 (500 bp)

TTTGAÄAAATTTTCAGTCACTGCAGAATTCGCATCTATTCCGCATTCAGG CTCTCCTGTTCTCACCTTGCCTTAGTGCGGATCTTCTATAACCACCACAG TGACGTTTTCAAGSTACTTTATTGAATAATAAGAAAAAAGTGCACACAAT CATGTAGTTAACTTTCTGTGCTCTTTGCCAGTTTGAAGGGACCCTCTTTT

TCCTGGGCCACAGGCTGCAGCAGCTCACCTGGGGGCTGGGGTCTCGCTCT GCGGATGGACCATGAAGGCCGGAGCCAGGTGGGGGCCAGACGGCAGGG CAAAGGGTCTGCACACACAGCGTCCCCCGACCCGGCTTCTCTGGGTTCT TGGGGGGTTGGCGAGACTCTCTCAGATTTCCTGGGGAACTTTCCA AGAACTGGGAAGTCTTCCAGAAAGTTGGGGTAGGGGAGGTACCCCCAAA GTGCTGCTCTCTCCCCATCCCCACCCCGCTGTCCATCGCGAGACCCC GGACCGCCGTCTCCCTGCCGAGGTGTGGGGTCCCCCCCTTGCCGGCCAG GCTGGGCAGGGGTGAGCCCCCTTGCTCTGCAGCTGGGACTCAGCCTGGC GAAGGCGGCCCCAGGAGGTCCTGCCTGGCAGCTGACCTTCACCCGC Contig 64 (500 bp)

### Contig 66 (500 bp)

TITGCATTCAGCTCGTACCCGGGATCCTTCCCGGGGGCTCTGGGGGTGGG

ATGTCAGGATAGTAACCTGGGGTGCTCCAGTGACAATGCCAGATCCTTAA CCACTGTGCCACAAGGGAACTCCTTGACCTAGAATCCTATACCCACTGCA AATATATTCAAAAAAGGTAAAGTCCTGAGCAGAAAACCAAAAATGGGAT AATTCATTTCTGGAAGACCTTCCTTGTTAAAGGAAGTTTTTTTGGACGTGA TGGANACGGAGCAAATANAGCTAAAAATAAAGTTCATCTCTTTCTCATTT TTTAATTGCTCCAAAAGATAGCTGACCTCTAAAGTAAAAAATAGTGGAAA TGTAGCATATGTCTCTAGCGTAATTTAAAGTATAACTTATAGCAATGATA GCCCAAATAAAGGAGGAATTGAGAATATACAGTTGCTGTGTTCCCATTGT GGCTCAGCAGTAATGAACCTGGCTAATATCCATGAGGATGCAGGTTCAAT CCCTGGCCTCACTCAGTGGGTTAAAGGATCCAGGGTTGCAGTGAGATGTG ACGTATGTCACAGACGTGGCTCGGATCTGGCATTTCTGTGACTGTGCTG TGGTGTAGGCCAGCATCTGCACCTCCGATTTGACCCCTAGCCTGGGAACC ACCATATGCTGCTGCTGTGGCCCTAACAGACACAAAATAAAATAAAAATA AAAGAGAGAGAGATATACCATTGTAAATTTCCTCACATGACACAAAGAG CAATGTGATATTATTTGGTATATGGTGATTGATTCAAGATGTATATCATA **ATATTGATTCAAGATGTATATATTCCTTTTCTAAAAAAGAGATTTATACA** ATAAGGCAAGAGTGAAAATAAAGTGGAATGCTAAAGAATAGTTAATCCAA AAGAAGGCAGAAAATGGGGAAAAGACATATAACAGATGGAACAAATAAAA AAGAGCTAATGAGATTGTAAAATTTAATCCAAACATACAGATAATCCCAT TAAATTTAAACACTCTCAACACATTGATTAAAAGAAATTGTCAAATTGAA TAAACAAAGCCAAGACCCAACTAGATGCAGACTATGAAAAACCCACTTCAT ATAAAGACATGGGTAGGTTTAGAGCAGAATGATGGGGAAACCATGTCACG CAAACATTTGTCAAAATAAAGCTGGTGTGGCTGTATTCATCTCAGACACA GCAGACTTCAGAACAAGAAACACTSCAAAGGATGAAAGAGATACTGCATA ATGATAAAGGGATCAATTTTCCAAGTGCAGGCTCCAAACAACAGAGGTTT Contig 71 (500 bp)

23/48

GACGTGCAGTAGCCATGACCTCTACGGCCCCCACTGACCAGCCCGTGTCC TTGTCCCGAGACCGACCCCTAAGCAATAGGATGCAGCAGAAGTGACAGAA CGGCCTCCGCGATGAGGTCGCAGAGGGCTCTGGCTCTGACTCAGGCCCCT CATCCTCGCTCTCCTGGAGCAGGGCCAGGTAGGGGCCCCCCAGAGACGC CCTAGAGGAGGTGACGGCCAGCCAGCCCCCCAGGGAAGGCCTGGGGAC ACCAGGGAACAGAACGGCACAGGCTCCTGGCACAGTCTCCCAGGAGCCCC CTGGTGGCACAGAAATCCTGACCGGCCCAGTGGAGGGGGGCTGGGGCGGGG CTCGGGGAGGAGGGACTGGGTGAGGCCGTCTGACTCCTGGCTGAGCGCCG CATACTTGCTGCCTGCCCACGATGCCGGGCCAGGCCTTCCGCACGGACCC AGGCTCACATTCGCCCTACATGCCACTGTGTGGGAGTTTGGGATGGTGTG CCCGCTGGGCCCGGGGTCAGGGCACGCTTCCCAGAGGAGCGGGTTCCAG AAGGCCCAGGTGGAGAGGCGATAGGAGGGCTCCAGGGGGCTTCCCAGGCC CCCTTAGCCCAAGGCTGAGTTGTGACCGCAGGGAGAGGAGAGAAGGAGCA CAGGGGCCCCTGGACGGCAGAGTCCCTGCTCCAGCTGCCGCCCCGACCCC AGGTCCACCTTCATTTCACAGCCTGGCCCCGGCCGCTCTGACCGGCCCT GCCCATGCAGGTGTAGCGGGGCAGTGAGGGCCAGGCTCCCAA Contig 74 (450 bp)

GCAGGCCTGGCAGCAGGAAATGATCCAGAAAGTGCCACCTCAGCCCCCA GCCATCTGCCACCCAGCTGGAGGCCCTCAGGGGCCGGGGGGCCA GGCGCTATMAGCCGGGCCGGCCCCAGCCGCCCCCAGCCCTCTTGGGACCAG CTGCGTTCCCAGGCCGGCAGGAGGAGTCTGTCCCCTTGGGAGCAG AGCTGGCTCTGGGCTGTCCTGCGGGCCAGGGCATCTCGGCAGGAGGAC GTGGGCTCCTCTCTGGAGCCCTTGGGGGTGAGGCTGGTGGGGGCTGCA GGTGCCCTGGGCTGCCTCAACGCCGCCCGGTCCCGAGGTCCTCACCC CCCGCCATGGGCCTGCTGACGCGCTCCTGCCCAGGCTGGGCCCTTGC TGGCCCCTCTGGAGCACCCCCCCCGGGCCCAAAGCCTTTCATGAACA Conlig 75 (1363 bp)

CCTCCAGCTGGGCCCGGCAGGGCACCGTGCCCCTCAGGGGACACCACGGG GGGCCACAGTGGCCTCTCCTGCTCCAGGCTCTGCTCCCGCCTGGGGCCCC CTGGGCCGCCCCATGGCCAGGGCAAACTCCCAGTGCGGCTGCCCGTC TGGGCAAAGAGGCCGCCAGGCCCCGCGTGGTCTTAGCAGGCACTGGCGGA TGCCGNTAACTAACCATTTCTTCCGCAGGAGTCCGAATCTGCTCTGACCA CGGGCCCTAAAAATCGCTCCTGGCCCGCAGAGGATCCCCGAACAGCGGGG CTGCCTCCTGCTCCTGCCGGGCCGGCACTCGGCAGGCACGTGCCCTC GTCG"CCCCAGTCTGTCAACCGTCCGTCGTTACGATCCCCAGAGTCCCA CGCGCGGGCAGCTCTTTCCACACCCCGCACGGCCCCGGAGCTGCCTGGGC ACCCAGATCGCCCCTGACGCCTTTGCTCCTAATTCTGCTGAAATACACAT AACGTCTCCTTGAACGTTTGTCCATTTTCACGGGGACAATTCTGTGGCCG TAGGTACACTCCCCTTGGGGCGCAGCCATCGCACCATCCGCTTCCAGGAC GTCCCGTCGTCCCAGATGGACACTGTCCCCACTGATCCCTAATTCCCTGT CCCCCCAGCCCTGCCCTTCCTGTCTCTGTGGCCCTGGCGCCTCCAGGGA GCCCCTGTGCGTGGGATCACAAAACGTGTGTCCCTTTGCGTCCGGTGTGT GTCTCTGAGCATCCGGAGCTTGGGGTGCTTCCACGCTGCGCCTGTGTCAG GACGTCCTTCCCTTTTGCGGCTGCGCGATGCTCCCCGTGGGGCTGCCCCA CACTGCGCGTGTTCGCTCATCCATCCACTAAGGCTGAGTTACTTTTGGCG GTTGTGAATACTGCTGTGTGAACACGGGCGTGCAAATACCTGCTGGAGGC CATGCTCTTAGGCCTCTCGGGGGGCACACCCAGAGCGGATATGCTCAATA AGGTAATTCTGTGTTTAGCTTTTTGGGGAACCATCAGGCTGGTCTCCAGA GTGACGGAGCATGCGTCGCATTCACAGGAATGGTGCTCGAGGCTTTGAGG TCTCCACCACTCGCTTCCTATTTTCTGTGCGTCACAGCCGTCGGAACGGC TGGGTGGTGCCTCTGTGTGGCTTCAATGTGCTTTTTCTTTTCCTGGCTAT GAGGTTGAGCGTTTTTTATGTACTTGCTGGCCATTCGCAGGGTTTTTGGG GTTTCTTTTTTTTTGCCTTTGGGGACGGCGCCCAGAGCGTATAGAAGT

TCCCTGGCTGGGGACTGAATCAGAGCTGCAGCTGCCAGCCTAGCCCACAG CCGCAGCAACGCA

Contig 76 (500 bp)

TCATGCCATCGCCACCGCCCCCCCCCGACGTTTCAAACACCAGAACCA CCCCTCGGGCGGCAGAGAGAGGACGGCAAGGAGAGACAGCCTGGTCCCAA GGCCTCGCCCGGTCCTGTGTCTCCGAGCGACATTTCTTTTCTGTTTCCCTC CTCCGCGGTCCAAGTTTCACCCATCAGAGGCGCATTGTTTTCATCATCTG AAAAAAAATCTCTGTCTCTTAATAAAACACAAGAAAAAGTAGCCTTCGA AAGAAAGCACATGAATGATATGTGCTGGCGACAGTGCTGGCGGCCTCTGA GCCGTGGTGGGAGCTGGGAGCCAGCGAGCCCCTGACCGATCACGTGACC CACGTCTCTCCTGCACAGCTGGCTGCACCTGCACGCGGTGACACAGGGAC CCAGCCTCCTGCCAGCAGGTCACCCCACCCGTCCGTCTCCTGTCGAAGG GGCAGCGTTGCCTTCTGAGGGTGGGCTGCTCTGAGGGGGCGTCCTTTGGCC

Contig 77 (626 bp)

TGCCECCAGCCGCCCGGCCCCGCCCTCCAGACAGGACGCCCGGTCACC
TTGCGGGGACAGCCAGCCTCGTGGCCTCGAGCAGAAGAAGTGAGAGTGGG GTGCACAGGGGCCCCCGGGGAAGGAGGGGACAGCGGGGGTGAGCGGG TCCTTAAACCGGGCCCAGCCTCTTGGGCCTCGACCCAAGGCTGTTTGGAA AATAGGTGGACCGTGGCCCTGACCCGAAGGCCCAGCGGGGACCCGAGTGCG GTCCCCAATGGATCAGCAGGCGCCTGGGCAGCCTGCGGCCCCGGGACCCG TGAGGACCAGCAGAAACCACGCCCTCCTCTCTCCCGTCCTCCCCCTCGC CTCCGACAGCTCCGACTCGGCTGCAAGGAAAAGGCCCCAGCCCAGCCCGC CGCCACCGGGGGGGGGGGGGGGG

Contig 78 (500 bp)

TACTCGGGTTTGTTACCACTGAGCCACAAAGGGAGCTCCTAAAAATAATA ATTTTCTTAAAGCCAATGACATGGAGAGCAGTTAGGGTGGAGGCTGGTGG GTGGTGGGGCCGCGCAGGCGCCCTGAAGGTCCTGAGTGGCACCCTTGGC CGGGGGAGGTGGGTGGGCGAGGGTGTTGACAAGGGGCCAGGGCCTCGTCC GGGCAGGAAGAGCCAGTGGCTCCCAGTCCCCTGACCTTGCTGCCTT GAGCCTGGTTCTCCCCAAAATTCTGTCTGTGTCCCTTCACTTCACGGAAG CTTGGGGCCCGTTGCCAGGGAGACAGATGGGCTGGTGACACCCAAAATGA GCCACCAGGAGGGGGGCACTGACTTTAGCCACCGGTCACATCAAGAAGC AAACAGGCCCCCGCTGCTGTAAAGGCAGCTTGGGGCTGGGGTCCGGGAG CACCCCTGGGCTGGGGAAAGGGGGTCCTCTCAGGCCCCCGGGGAGGATG Contag 79 (427 bp)

TCTATTCGCCGTUGCCGGAAGAGGCTAACCGTACATTGACCGGGCATCTG GCGATGTATCACTTCTCCCAACCGAAACTTCCCGGUAAACTTGCTGCG TGAAAACGTTGCGGATAGCCGAATCTTCATTACCGGTAATACAGTCATTG ATGCACTGTTATGGGTGCGTGACCAGGTGATGAGCAGCGACAAGCTGCGT TCAGAACTGGCGGCAAATTACCCGTTTATCGACCCCGATAAAAAGATGAT TCTGGTGACCGGTCACAGGCGTGAGAGTTTCGGTCGTGGCTTTGAAGAAA TCTGCCACGCGCTGGCAGACATCGCCACCACGCACCAGGACATCCAGATT GTCTATCCGGTGCATCTCAACCCGAACGTCAGAGAACCGGTCAATCGCAT TCTGGGGCATGTGAAAAATGTCATTCT

Contig 80 (650 bp)

GGCGTTGCCGTGAGCTGTGGTGCGGGTCACAGATGGGGCTCAGATCCCGC GTGGCTGTGGCTCTGGCCTAGGCCGGTGGCTGCAGCTCCGATTCGACCCC AAAAAAGGAAGAAAAGAAAAGAAAAGAAAAGAAAAGTCAAAAG GAGCTCCCCTGAGCGATGTCTGTCTACGAGCAGGTCCCTGGGAGCCTGAG GCAGGGTGAGCCTGGACCCCTGAGGGCCACTCCAGACTCAGTGCTCTCAC GGTCAGAGGGGGCTTCAGGCTGCAGGGCCAGCGGCAGCTCTGGGCCCG GGGCGGGGGAGATGGCCTGAGGGCCTTGCGGGGGCTGGAGGGTGGGGG GCTTCCTGGAGTGGGAAGACGGGAAGCCAGGTCAGAGGAGGAGGAGCGAGG CCACATTCAACAGCCACCCGGCCTGTGGTCCTGGCAGGGTCCTGGCAGAA AAGCCCCAAGGGCCCCAGCCTGGCCCTCTGGGCCTAAAGAGCCAAGCCCC Contig 81 (550 bp)

TTAACCCACGGAGCAAGGCTGGGGATCGAACCTGTAACCTCGTGGCTCCT

CGTCGGATTCGTTAACCACTGCGCCACGACGGGGACCCCCAGGGCTGGC
GTTTCCCTCTGTGTGCACACAGTGGACCTGAGCCAACCAGCAGGGCCTTC
ACCACCACGGCGCAAGAGTCGGCAGCAAGAGAGCAGTTCTCATGGCTCA
CTTTCTCCCCCTTCCCCGGAGTGGTGACAAAACCCCGCCCACCGGACT
CGGTTAGACAAGGCGGTGCCCAGTGCCCCCTCTTCTACCCGCACGGCAC
GGCGCTCTCCTTTCTTTCTTCGGGGCTCCACCACGTGTCCTCAGTTTCCGC
ATGAGAGTACCGCGGCTGGCGGGGTGGTGGCTTTGGCCCATTACGCGGGGGC
AGGACTCCACATCACACGCTCTCTTTGCCCCATTACGCGGGGG
CAGACTCCACATCACACGCTCTCTTTGCCTCTTTTGCCTGACACCATG
GACTTCAAACAGGAACAGCCGTGGAGGCATTGCAGCCCAGGGCCCGGGTT
Contig 82 (550 bp)

CTGAGCCCAGCTATCTAGATTAGACCCCGGTCCGTCCCAAATTCTTCTCA AAGCTGTCCCGAGATGAGAGATGAGGTTTTCGTGTCCTCTGCTCCTCG CTTCCCCTGGGATGTGCCCTAGGGTGGGAGAGGGTGTGTCCCAGGGCTCA GCAGGCGTCCCATCTTCCCGAGACGGGAGAGATCCCCTCCTTCTCGGCG CCTGTCCCCACGGCCCCCACAGACACCCCCCCCCCGGCATGGCACCCAT GCACCTGCCATCGTGCCCAGTAGGGGATGGGTTTGGCGAGACTGGAGATG GCTGTAGCCACTGAGACATGCCCTGCCACGTAGCCTGACCCCCTGGGTGT GCTCTGTGAGATCTGGGGACCCCCAGCACCCTAGGGATCATCTTTGCCA GCCTCCTGGGGAGCCTCTCAGAAATGGGGGCCCCCAGAAGGCTGGCAAAG GTGATGGGGAGCGTGGGAAGTCTGGCGGGTTGGCGGGGTGGGGGGGCA GTGCGGGCTGGGTGGGGGTGCTCCGGGGTCGGAAGTGCTCCAGCAAGGT TTTGGAUACAAAGTCAGGAGGAAGGAGTUACGAGAGACTTGCAGAATTA CAGGTAGAATCAGGAACCCACATCGACGCCAATTGATCTATCCCCCCCTT TTAATCCCTCCTTAGCTTTTTACGCGCTCAACACCAAATTAAACGTACTC CCCACCCACGTAACAGGGGGGGGGGTGACCCGAAGGACGAGGAGCACACG AAGCCACCATCCGTCACCTTGGCGGCACCAGCCGCTGTCCTGCCCTCCGC CCAAAGATTTCAGGGGAATGAAACGGCTGCCGCC

Contig 84 (550 bp)

TGCCCCTGACAACCCTGCCCTGTTAGCCACACTCGCGACTAATAAGGCCA
GAGGTCAGCGGGCAGCCCCACGCGGAGAAAGTGCCTCCGTGCCCCCACC
CCTGGCTCTGATGGCCCACCCTGCCACCCCAAGGTGGCCTCGCCTCCCACC
ACCTCCAAGGTCCAGGCGCATCTCCAAGCACCAGCAGAAGCTTCTCCAGG
GTTGGTGCCTGCTCAGGGCAGAAAGCAGGGTTGAGGCTCCCCAAAGGGCC
ACTGCCACCAATGCCCCCCCCAGCAGACCCCAGCGAAGGGAAGGCCCACCC
CAGCCCGGGACCGAGGCCTGAGGGGACTTCGAAATCTTTCCCGGGGGCC
AAGGGCAGCAGAGCCCTCCTCCGGGACTTTCAAAATACGGCCCACCCCA
AATTGCCACUTGGCCACAGAGCAAGGACTCGCTGCCAAAGTGCCCTGC
TTCAGCGCAGGAAGTTCCCCTCCTGGGGCCTCCCCTCTATAGGCACAGG
Contig 85 (500 bp)

TGAGCCAGGGCCTGGCCCAGCTAAGCCCCTGGAGCCCTCCCGGCCTGTTT
CCTGCCTCCCATGCTGGCGGAGCTCGGCTTACTGAGCGGGGGCCAAGGCCA
GTGTGCGTGTGGAGGTAGATTCCACTCAGCTGGAGGTTGAUGTTGGCAGG
GGGCCGCAGACCCTCAGGCCAGCTCTGGCCGGCCAGGTCCCTGAAGCTCC
CCCGGCTGGCCTCCCGTCCCTGCCTCTGGCCTTGTCCTGGCCCTTGCCT
GACAAGCTTCTGTGGCTCTGCCTGCAGAGAGACACTTGGCTCCCCGGTC
TCGGATGAGGACGGGGCTTTTCTGCACAAGTCCTCCCCCAGAATGTTTGG
GGCGCCAGCAGCTGAGCCCAGCACGTCTCCCCCTGCCTGGCTGACAC

GAATCCCGGCATCGAGGCGGGAAGGGGGATGGAGGGATGGGGCCTACCCA CCCCTGCTCCCCACCAGAATAGCTGGGCGGCCCCCATGGGAGGCCGCCC Contig 86 (913 bp)

CTGTTTTCACGTCTTCTGAGGACACACCCAGAAGAGGGGCCTCCAGGCGCCCATGGTGACTCCATGTGTTCACTGCTGAGGCCTCTGCAGACCGTCTCCCG CAGCAGCCGCACCGTTTCCATGCCACCAACAGCGTGCGAGGCCGCACTG TCCCCACGGCTGTGCAACTGTTTTGAATCTGAGTTATATAAGCAACAGAC GCTCCTTCAAACACACTCACGTGCACACGTGCGCACAGGCGCACAGACAC ACACACGGAGTAATAGGCCTCCCCCCCCCTCAGCCCAGAGGGGGCCT GGGGCCCTGGAGCCTGTGCTTTAGGGCCTTTTAGGAAAGCTGGTGCCTCC CAGAGGGGCCGCCCGAGCGTTGGCTTCCCAAGTCCCCACCAACCCTCGA CAGACTCAAACGTTGGTTTCTTTCGTGCTTTTTGCCCAAGGGATGGGCCCG 'AGGTGGCCCTGCCTGAGGTTTCAGCCCAGGCCCCCAGGCACCCTTTCTCT CCCGGTCCCCGGCCACTTCATGGGACAGGGGGCCTTCCCCCACGTTGTCC CCTGGGTTGTCGTGCTTTTCGTAATGAGACGGAGGCAGCTGCACCTGTCC TGGGGTGAATTCTCTTCTGCAGGAACTCGCTTCCCCGGCGCCTGGTCTGT CTGTTCCTCGGTTGTTGGAACCTCTCGTCACCAGAAAGGGTGGCTCTGAC GTCGCCCTTTCCCTCCGTGGCTTTTGCAGTCTGGGGTCTTGTCGGGGAACC TGCCCCAAAGAGGGGAGTGACCCCCCACGAGGGAGACGTAGCTCCTGTGG CGACAGCACCGGGGGCCCCCAGATTCATGGGGTTCACGCTCACAGTCGCA TGACGCTGCCTTTGGACGAGGGGAGGCTCAAGGGAAGCTTCTTTCCTGCCA CGAGCCACAGGCA

Contig 87 (650 bp)

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FIGURE 6, CONTD.

GTCTCCGGCAGGGGTGGGCTCTGAACGTCCAGCTCCGCAGACAAATCAGA TTCCCCCGAGCCCTGAGAAAGCCCCCTCCCCAGCCCGTCTCCCCACCTG TCGGTGGACAGAGTGACCCCTGCCCGGGGCTCCCGCAGGA GATGTGAGAGAGTAAGAGGCGGTACAGGACGGCCGGGGCGGCCCGGGCGA GGTGCAGGTGTGGGTGTGAGGCTGGCACAGCCTCCCT CCGAGCTACAGATGCCTGCGCATTCGCCCCAAGTGTCCTGGACCCTGGAG CCAGGCAGCCCACCCGCTCAGCCTGGCCAGACCCAGCGTTGCCCTTCACG CCCTCCTCCCCCCCGGGGTCCTCGCGCTCGTCTCCTCAGGTTGGAAGC CCCTTCCCACCTGCCATCTTGCCTGCGCCCAGGATACACGGCTCAACTCA AGGCCTCACTCCTCGCCCTCTCCAAGGCTCTGTCCAGGCCCCTCTCTGAC CTGGCACCACCTGCCGCCTCCTGGCAGCCCCAGCAAACCCCCTGCCACAG TCCACGACAGTCCTCTTCTGGCTCTGCCCCCAGGATGCTTCTAGAACTGG GGGGGGGTCCTTCCAGCCCACGCAGCATCCACTGGGCCCTGGGCTCCCT CCCCAGGTGCCCCTCAGAGCTTGCAGCTGGTGCAGACGGCTCTGCTCCGA ACCCATGCTCCCTGCGCCCTTGGACCTCGTGAGATGTTCCAGGTCATTTG GC1'GCACCCAAAAGAGTGGCCCCTCAGGGT'CCCCCCTGCGCCCCTCCATC

#### Contig 90 (350 bp)

GTACTGTAGGGCCTCATTCGAATAGCCTACTAGGTCACAGCTGATCCACA
CCTTAGGCCATCACAACTTCCCAGAGGTACTGCCGCTCCTTTGATC
AAGACGGTAGTGACTGCTGTAGAGGTACTGCGGTCACTGACCG
AGTGTGGAACCCTGGGGGAAGGCTGTGGGGTCCCCGGCTGGGTGGCCA
TGTCATGTGCCCCTTTCTATCCCTTGGACGAGGCTGGTTCACTCGGCTCT
AGAGCCCCAAGCCCCAGCTCCTCCCCAACCCCCCAAGCCTGAGCCTCAT
CAGACCCACCACCCCCATCGCCATGCTACGCAGGACACACCGCTCTCCAC
CCCCACCAGCCCCCCCACCTCCCCGAGGTTCCAAAGCTTGA
Conlid 91 (1464 bp)

TCCAGGACCTGATGCAGCAGCCACGTCGCGAGGCCCCTCCCACGAGGCCC CTTGTTGACCAGCGCTAGGGAAGGGGACCAGGGAGATGCTGAGAACGGGG CCTTCCGAGGGGGAGTGGGACTGACTGTGACCCAACACTCCCCACCCC CCTCTCCCGCTCCAGAGGGTGCCAGCCTGGAAGCTCGCAAAGTCCAATCC ACAGGTGGGCTCACGTGGGGGGGGCTGGTGGCCCCCACCTGGTGGGGCCCC AAGCTGCCTCTGGGCGGGGTGCGGGGCTCCCAGCAGGGTCCCATCCAG CTTCTCCCTGGGGAGACTCACAGTTCTGGGAGAAGGGTCCTGACTGCACC TGACTGGTCTCCGCATTTGCCCAGGCTGGGCATCTGCCCAGAGGATACGT CCAAAGGCAGGGCAAAGCCGGGCCCGTCCCCCGGAGCTCCCCACAGGCGC CAGCUGGGTGGAGCGAGGCCTGAGCCAAGGTGCACGCGAGGGCCACAGAAG GCCGAGGCGGGCAGGAGAGAGCCCCAGCCTGGAUGGGGGTGGCTCCC CTGGGCAGGTCTGGGGCTCAAGAAGAAGAGAGTGTGTGTCCAGGGGGCTG TCCAAGCTGCCCGGGAGCCTGCCTGCCCACCTCCAGGGAGCAAAGCAGGG AGGCTGCAGCTCCCCGGCCGGCCGCTCTCCAGGACCACGCGTGGCCCAG GCCTCAACGCTCCTCCCACAGCCCAGGAGACCCAGGGCACCCGGTCCATT TACGGCGCCTCCGGGTCCGTTTGCCTGCGCCCTGGGATGGACTGTGGGG GCGGGGCGCTGTCTGGGGAGGAGGAGGTGTCTGAGGCTGGACACCTTGA AGGCAGGTGAGAGTGACAGGTCCGTGCGCAGCAGCCTTCGGCTCTGGATT CTGGCCCTGAGCGAGGGGCTGGCTGGAAACTGGGCCGGGGCTGCCCCAGG AGAGTGTGCAGGGAGAGGAGACGGGGTTTGGCCCCGGAGGTGCCGGGGTG GTGCCCTGGAGTGCGGCTGAGCGGGAAGTGGGTGTTGGCGTCTGGAGACG GGGGGTCGTGGGCTTGGGATGGTGACAAGACCCCCCAGGTGGAGGCGGCC GCAGAGGAGGCAGAGAAGCCAGGCCCCACGGCGGGAGGCCTGGG AGTCAGGAGGACCAGCAGAGCCCTGGGCTCAGTGTCACCGGTCCTGGCA CCTCGCCGACGGATGTCCTGGCCGTGCAGTGGTTGTCCCCTCACCCTGAG CCCTGAGAACCATGCAGGATGCTGGTGTCACAGCAGGAGAGGGCCAGGGC CTGGGGAGGAGTCTTACTGGAAGCCCTTCTCCTTCCGTTTGCAGCAGGCG GGAATGACTGGGGG

Contig 92 (694 bp)

ACTTGGGCACGGCCAGGTGGGGCCCGGCAAGGGGGAACAAGGACGCTGGC CTCCAAGGCCCCACGTGGGCACAGAGGAAGAGCCGACCCAGGTTGTGGG CGCATGGAACCCCCCACTCTGGGGGCCAGGAGGCCGAACGTCCCAAGGGC GGTGCCCCCTGCCACTGGCGGCACCTCTGACCCCAACTCCTTGCGGGTG GACGGTGGATGGATTTCCTGCAGCCTTTCTTCTGGAATAGTCTCTGCCAT CCTCGGGGAAGCAGTGATTGCTCTGCCCAAGTCCAGGCCCCGCCCTGCAA GGTGCCTCCCACCCCAATGAGCCCCCGGACAGTTCGAGGGCTTCTCACGC "ACTGAGGGGTATGAACAGCTGTCCCCCTCGGAAAGTGGGGGACAGGCCC CTGCCACTCCATCCTCGGGACGCCCGGTCTAGTCAGCACTTGTCTCCCTG CCTTGTGCCCCCCTGACCTTTTTTGAGGACCATCAAAACCTCAGCCTCTG CCCCAGGAGGTCAAGCCCCCCGTCCCCAGCCCCCAGACCAGCA

Contig 93 (900 bp)

CCAGCCCCATCCCCGGCTGGTCCCCCACCACACAGAGCCCCCGTTTCCC AGGGGACAGCACAGCCTGCCCCCAGGTCTTACATAAAGTCACCTTCTCAG AGCTCCTGTCGCGGCTCAGGGGAATGAATCTGACCAGCATCCATGAGGAC ACAGGTTTGATCCCAGGCCCCGCTCAGCAGGTTAAGGATCTGGCGTTGCC GTGAGCTGTGGAGGTCGCAAGACGTCGCTCAGATCTCGTGTGGCTGT GACTGAGGTGGCGGCCAGCAGCTGCAGCTCTGATTGGACCCCTAGCCTGG TAAATAAAAGAAGTAAACACACCTTCTCTAGCCATAACCACCTGCCTAGG GGCGGAGGCCAGGAAGUGGCACCCCCGGCCCCAGGCTGCCCGTGCGCCC CGGGCAGGCGGCTCAGCCTGCTTTTGTCTGTGATGTGAGCCGCCCCAGC CCCACATGGAGGGGCTGGGCTGCGCAGTAACTGCTTTAACTGACGGGAGC TTCGACCAGCAATTCACCAGCGGGCATGCAGCCGGGAAGGGAAGTTATTC CTGTGTAGCTATTAGGCGCCGGACTGAGGGTGTGCCTCGCCCTGGGCCCA CCCCTGGGGGGAGGCATCACAGGGGTTTTCAACACCTGCCCATGAACACG TGTCTCTGAAATCCGGGGAATCCCCACTGCAGGCATGTTCAAAGGGTCAA GACCGGGGCTCTUCCTGAGAAGGACTGGCGAAGGCCAACTACAAAAGCGC ACCCCTCTGTGCAAACCCCCAACCAATGGAACAAAACTCCAGAGGGGCCA Contig 94 (550 bp)

AGTCTCGGCTGTGCCATGGGGTTGCCAAGGTGCCAGGCAGAGACCTTGG GGACA/AGUTCCTGTGAGCAGAAGGACATGGCCACGTCCCCTGCTCAGCA GGGCCAGGCCCAGACACCCTTCGTCCCTGCCGGAGTTGTTTTGCCCTTCTG
TTCCTGGAACGCCCCCCTGCAGGTACAGGAGGCCCCTGGGGCTGACGCTG GGGGCTGGACGGAGCGGGTAAGACAGAGAGTTGACTCTCCTCGAGTCT GTGCAGGGCTGTCCCCGGCTTGGGCTTCGTCTGCAGGGCCTTTCGGGTCA GGGTGGCCTCAAGGTGACGAAGACCTGGTCCTCGGGAGTCTGCAGGCGCA CAGGGAAGTCTGGGGCCTGCAAGGCCGTCCGGGCTGGGGAAGGCCAAGGT

Contag 95 (1200 bp)

GTTTGCTCTCAGCAGGCAAGGGCCTCCGAGGCCTTAATAGCCCATAATGA CAGCGCCGGCTCCTGGCATGGGGCCCCCCCCTGGCATGGGGCAGGGCAGGG CAGAGCAAGCAGCATGCAGCTTCTACCTTCTTCCTGACCTCGTGGCCCCT TCCGAGGCCTCAGGGGTCCCCCGAGTGGGACCCCAGCCCTGGCTCTCCT CTCCAGAGCCAGGCCCAAGGCTGGGAGTGGCCCAGAGATGAGGGTGCCCG AGCAGGGCACTGCCTTGGCGTCCCCATCCCTGGCGCCCTCAGGGCCGTACT GTCCAÁAACCAAAAGAAAGCAGTCAGCAAÁACTTCTCCCAGCAAGCTGGG GTCAAAGGTCGCTTCCGAGGCGTGATCAGGGTGGGCTTTGCTACTGTCAC CGTGTGCCCTGGGAGAGGCACAGGGGACACACACACCTCCGAGAACC TGGGGCTTCCAGGGCGTCAGGCTGCCTGGGCCATCCLGGGCCCCTGTGGT CCCAGGATCTGCCGGGACCGTGAGGCCTGCGTCCCACCCTCTGCCTGGGA CAGGCCCCACAGAGCTCACAGCCAGGGGACCGGGGACAGGGCCCCGCCTG GGCCACCTGCCTCCAGCCTCACCCAGGCCTGGGCCCCAGGCCTGTGCCTGC GACACCCTGAGTCTCAGGACGGGCGCGGGACAAAGCCGCCCUUCCCCTCC CCCGGCTGGGAGGAGACCCGCGTGGCCCTGACGTGTGGGCCTGTCAGAGC GGAGGCCGGCGGAGGGGATCCACGAGCUGAGGGCCCGGAGCTGCCCACCC CACCGGTCGATTCCAGGCACTCAGGGATAATTCGGTGTTTAGAAGTCAGG TTAACAGGTGCCCGAACACGCAGGTCTGGGGAGATGCTGAGGTCGCCAAG

CCCCCTTATTTTAAATTTCCGAAAACAAAAACCACACCTCTCCCGTCC CCGAAATTATTTTGGTATAGTCTTATTCAAAGAAGTCCTGCCACTGAAGC CCACTTGTCCTGTCCCGGGCTGCTTTGGCCAAGGGCCCTGACGGCCCAG GGTGGCTCATTCCCGCATCCCCGCAGAGGCCGCCTTCACATCCCATGCGG GAGCCTGGCTTCCGGCACCCGGCTGTGCCCTCGCTGTGGCCATGGACTGC TTTCGCAGAAGCATAGGGGCCACAACATGGGACAGCCTCGCTCTGCTCGC TGTGGTTCCGUTGAACCTCTCAGCTGGACATCTGGGCAGCAGCACCCCA GCTTTGCTTCAGGCTCTGGTTCCAGGCTGGGCCCTCCTCGGCCCTGCCCG CTGGGTGCCAAGCAGGGCTGGTCCCGGCTGTGCCCCCGGGTCTATAGAAGC CTCTGCAGGGCTTCCTACAGCCAGGCTGGGATTCGGCGGCTGCCCGGGAC TGAGGCCCCCTCTGAUTCTGACCCCCCATCCTTCCCTCCCACACAGCCC CCCGCCCCGCTTCTGCTTCAGTGAGGCCCCACCCTGCCTCACTCGCTGA CATTTCCAGAACAGGGGGTTCCAGGAAGCCCTGAGCCTGCAGGGGACTCA GTGACCAGCCGCATCTGAATTTTCCCTCCTTCTGATCTCTGGAGACACGT CTGGCTCAGCCTGGCTCGAGTGCCCTGAGCTGGGGACCAGGACAGACCTG CAGATGGAGGTCTGAGCCTGGGCAGGGCAGGGCCCAAGGCTCAGGGAGAA ATTGCAGGTGTGAGATCAATGACCGGAGCCTGGATGGGGCCGCCCTGGCC AGGGCACCTTTCTCCCTGCAGCTCCCTGCCACTGTCCCCCCCAACTCTGG GCTCCTGCTCTGGACCCAGTTGTGTGTTCCCCTCCTCCCAGCCGAGCCAC CCTCCCCATTCTGCCCCCCCAATCCAACACCCTATCGTGGGAACCAGT GGAGCTGAAAGAAGGACCCCCCAAGGGCCCCCCAGCCGCTGTAATCCTTG GGGGCCTCTGCCCAGGTGCCAGGTCTCGGGCAGGAGGGGCCGCGGGCACA GCCGTGGCAGATGCGCCCCCAAGCCTGGGCTCGGAGGAGCCCCGCCCCC ACTGACATTTCCAGGCCGCCGCTGCAGACCCGGCTGGCCGTGATATTTA GACAGGGCTTATTTGCCGTGACTGGTTTTTGATGACTTTGGGGCCCAGGA TGAGCTCAGCCGAGCCCGCGTTGGCCCACCTTGGTCTCAGCTTGGGTTTG ATATATACGCGTTCAACTGAACCGCTGACGCCTGCGTGGGCCGAGGCC Contig 98 (1354 bp)

GCTTGCAGTAGTTCATCAGATTGGACGACTCATAAATGTCAAGACATCTA AAGATTGGTGCATCCAATCATTTCCCACCAGGTTGTTTTTTTGTAGATGT CAACAAGCTGACCCAAAAACTCACGTGGAAATGCACGTCAACTGGGAGAG TTGAAACANTTTCTAAAAAGAAGAAGACGTCCTGGGAGGACTCTTCGCG CTCTTTGGTTTCGCTTCACTTTATATTATTAGTTACTGATTTTCCTAAAA GGTTGGGACAGAATAGAAAGCCCAGAAACGGACCCCCGCAAATGTGGTCA ATTGAGTTTGGGCAAGGATGTGAAAGCGGTTCAGTGGAGAAGAGTCTTTT CAAGAAATCTCTGGTCCTGGATCCACTGCTCATCCAGGCCCAAGAGTGAA CTTGGCGCACATTTCTCACAGTGTATACAAAAACTGACTCAAAATAATTC ACATACCGTCGTGTAGCGTATGAAGCCATGAAACATCCAGAAGAAAATCT CGGTAACCTCAGGGCATCTGGGGCCTCCACCCTCAGCACCACTGGCCTTG GGGCCAGATACTTACGTGTTCTCCTGTGCACTGTGGGACGTGCAGCCAAA CCCCAACAAGGTGACCATCAGAAATGTCTCCAGACGTCGCCAAATAACTG CCAGAGAGCACAGGAGCCCCTCACTGAGAACCACAGGGTGGGGCAGAGAG ATCTCAGACATGACACGATTAGUGGAAAACAATCTGACACACTGGCTTTG TTAAA'I'TTAAAACTTTTCCCCTGTAAAAGGCAATGGTAAGACATTAAGAG GCGAAGTGGCAGACTGGGAGAAAATATTTGCAAATCATGTATCAGATACG

#### Contig 99 (1000 bp)

GGTTCTCAGGCGCACGGGGCAGAGGCTGAGGGTCCGAGGGGCTTTGGGTG CTGGAAAGCCTGAGTTTGAATCCCAGCTCGGTTTCTTAAAGCTGTGTCTC CACGGCCAAGGAATGGGGCCTCTCTGGGAAAGGTCTGGGGTGAGGCTGGC GGGACCTGCCAGCCCGGAGGGCATCTGACCAGACAGCTTCTCAAGCTCA CAGGGCTTCATGGCAGGATGGGGAAGGCTGTGGTGGGGAGTGGGGAGCAC TCGACACCCTGTCCAGGCCTCTTCAGTCACGGTGGCCTCTCAAAAGGGGT TOTOTGTGTCCAATGAGCAAGTCTTTGTCCGGGGCAGGATTACTAAGTCC AAGGGTGTCTGCCCCTCCGTCGGGCACAGAGCAGCCCCCAGATCACGT GGCTGTAACTGCCAGGTTGCAAAGCCTGCCACCATGTCCCACTGGGTTCT CCAGTTACCTTGGGAGGTGCAGGGTGGCGTGATGGGGAAACTGAGGCAGA GAGCTGGCAAAAGAGTGCCGGCAGGGACTGCGGCCCAGACCCAGCTAA CCGACCCTCACACGGAGCTGCTTCTACTTTGCAGCCTGGACGTGGGAAAA GGTTACCCCACAGCAGCGTGTGCAGGCACGCTGGTATGTCTGTGTACTTA TGCATATGTTCTACGTGCATGCACGTGAGTGTGCTCTGTGCATTGTGCCT GTGTGTGTGTGTGTGTGTGTGTCTATACGTGTGTGTAG TGAATGCTTGTGCATGTGTATTTGCATGTGTATGTTTGTACGTGTGCAGT GAATGCATGTGTGCAGTGGCGGCATGTGCGTGTCCGCATGTGTCTG TITATACCTGTGTGTAGTGAATGCATGTGCATGTGTGTGTTTACATGTGC ACGTGAGAATGTGCACTCGTGCATGTTTGCATGTGAGTTTCATGTACACA TGCTTTTAACGTGTGCACGTGTGCACATGTGTTTCTGTGTCCCTTGCACG Contig 100 (1500 bp)

CGTATAAATATATAATATAGATAAAATAGATTGATAATAGATAAAC TAAACCCATTATCAATACCGGGTGGCCCCAGCAAAGGATACTAGCCAGTT TATCAMEGTGCTAAGTCAGCACATAGAATGGCCACAAACGAAAACCTGTA CTGCCTATGTCCACTCTAATGGAGTATUCCACTGACATCAGTGGTAGGTG AGCTGAGTCCATCTGGGCTCCCAGTTCGGGCCCGGCTTGTCCCCCAACGG AGGTTCCTTCCAGGGTTCCCCAAACCCAACCGGGCCCCCAGGTCTCCCTG TCTTGACTCGTTTCTGGAGTCTTCTGGGGCTCTGCAGTCCTCCCTTGTTG GGGCTTCTGTCCCCCTGCCCCTGGCCTTGCGGGCTCGGCCCTGCCCTGGG AGGCTGGGCCGGGCCAGGAGGGAATGCGCCTCACTCTGCTCCAGATGGAC AGGTCGGGACATGCAGTGGCCTCGCCTTGGGCTGCTGAGCCAAGAGCAGG CAGCCGCCAGCATCTGTCAGGGCCGCTGCAGGCGCGGGGAATGACCTCGA CTTCTGCTTGGCACCCAGCTCTGGAACAGCCCCCTGCGGAGCCTCCGCCU AGAGCTGGGCCAGAGGGTCCCCTGTGCCGGGGACCCCAGCAGGGCCCCTC CCTGACTCTCCAACCCACCTGCCTGGGAGGAGTGGCCCCCTGGCCTCCGT GGATCTCTGGGTCGGGGCTCAGCCGGCTTGACAGCCTGGGAACAGCCAAT GCACATCCCCAGGCCTGGCCACACCCTTCCACCGGGAGCGGGGGGGATCTG CATTTCGUCAGGCTCTGCGGGCAGCTCTGAGAGCCCCGGGTCTCGGAGCC CAGCCGTGGCCGTTGTACGCCCTGGGGGCTGTGGACAGCGTGTCCTCATT GCCCCTCCGAGGTCCGGCCCAGGTCCCCTCCCACCTGCTCGCCCAGAGCC CCGGTTTCAGGACCTT'IGCACGTGCCGCTTCCTCTGCAGAGAAATGCCTG GAGCAGATGTTTGTCCGCACGGCTGCTCCGCGAGGCCTACCGAGAGCCCC TCACCTAAACGGCCGGGCCTCAGCAGCCCGGGGCCCTGTCCCCACCGCCC AGGTGGTGGGTTCTCCTGTGCCAGTGTGGGCATCTCTGTAAGATACCTGT TTATCTGCTCATCGTCTCGGTCTCCCCCAGAAGGTAGAGCAGGCCCGGCA CAGCCGTCCTCGGGGTGGCCACTCGCCCTTGGGGCTCAGCCTCCATGCAC GGAGGGACGCCTGGTGACACGAGAGCCCCGTGTGAGTGTGCCGGGCCGCC AGCCTGCCTTAGGTCACAGCCAAAGCCGGCATTAACCACCAGGCCCTCGA

Contig 101 (600 bp)

AGTATATCGGGTGAGACTGGGGGACCGGTCTGCCGGGAAGCCCCACCATAA AGGCCACGTTGGGCCACAGTCCGGGCCACGTGAGTGTGGGCGGGTCCGCG GGTCTGCTCTTGGAACACCAGGATCTCTAAGAGGTACCAGCCGAGGCCAA CTTCACGTGAGCAAGTGAGCAAATGACTGAATGAGAGCCTGAGCGAATGA GTGAGGGGTGAGTCCGTCCACCACGCAGCCTAGGCTCAGCCAACCGCTGT CCCCGCGTCTCCACTGGTGACCAGAACGGAAAGAGTGGGT TGTCTCCCACACCCAGTCCCCAACCCCCCTGCACGCCCCACCCCTCCAG GGGTGCCGGGCCTGGGCCCCAGTCTGGAGGCTCTGGCACCTTC CTCATCCGTTCTCCCAGCACCCCAGGTTCGTGCTGAGCCCTCCTGGCCCA CAGGCCTCGGGGACAAAGAGGGCCACCTGGAGGCTCAGGGAGCCTCACCT GCCTCGTGGTCCTGGCGGAGGCGGGTCTGGACATGTGATAGACCGGCCTG GGCTCAGCAGCTCCTGCTGGAAGATGTCAGCGACAGCCTGGGCCACTCTC CCACCAGGAGAACTTATTCCTCGGTGGGGTCCCCCGGGGAAGGGATGGG ATCCCAGCGGGGACCCCAGAGCGTCCAGCACACGGACCTGTCCCTCCAGC CCCTGCCCACACGGATGCTCACAGCTCAGCCTCGAACACGCACCTGTTG GACTTTGCCTCCTGAGGCTGTCTTCTCAGCCGACGCGGGCCTCCGCTGCA TGGTCTGGAAGCCCAGTGGGACTCGCTGGTGACAGGGAACAGGGGCTCTT GGAGTGGGGTGCCGGGGGGGGGGGGGGGGGGGGCTTTGATGG CTGAGTGGGCTGAAGTCAGGCAGGCTCCCCAGGCCTCCCTGACCCCCCC CACCTCAAAAAATCCAGAGCATCCTTTGCTTTGGGTCTGGTGAGGCTCTC TGAEGTCAGACCCTGCGTGGCTGGCCAGTGGCGCTGGAGCAGGAAGAAA GCAGGACACCCCCCGCCCCTGGCCCAGGACTCCCCAAACCCAGCAGGAGAC ACCTGAAACGGGATGGAACCATCCTGAAAAGAGCCACCTCCTCCTCCTTA TGCATCAGCTGCCGGGGTCTGGGGGGCCCGCCCCAGGCCCCAGATGTCCGG GCTGCTCCCGTCTCACATCCAGGGGTTTCTGGGCCCAGGACTCTGTCCCC TGGCGCCGGGGACAAAGCCGGCTGGGGTCTCAGGTTTGGGTTCAGAGCA AACGTTGATCTGACCT,GGTTCTGAGATGCTCGGCCCGATGCTGCGTTGTC CCGGCCAGCCCCACGGAGGGACGCAGGGTGGCTGGCGGGGTCTGGGGGGCC CCTGCCCGCACCAGAACGTCTGGCTCAGCTTTTTGTCCTCGTGACCCATC ACTAAGGGCCACCCTCTGACCCGGAGCCCTGTCTCCGAGGTGGGAATTGG GGGCTGTCCCTCGCGTCATAGGACCTGGTTGGGGGCATCCAGGGCTGTGT CATGCCCCTCCCCAGAAGACTCTGGGGGGCTGCGGGAGGGTTTCCCCAGCT TCGGGCCAGCCTGGGGAGGCCGGAAGGCGCTGCAGGCCTTGCCTGTCCCA GGGAGCATGGCTTCGCTGCAGACTGGGGCCCCGCACACCCAGCCACCACC GGCCGTCTGGAAGCACT

Contig 103 (630 bp)

CGATGTGTCCCTGGATGCGCAGATGTGGGTACTTCTTGGACTCCACGGTC Contig 104 (1630 bp) GGTGTTGTCACTGCTGTGGCTCAGACCCCTGCTGTGGCACAGGGTCCATC CTTAGCCCAGAAACTTGCACATGCCACAGGTGCAGCCAAAAGAAAATTCT TACTAATAAGTTGTTCATTTGCCTTTACGTAGAGTGGCATCAAACAGCAA ATTTAAAACACCATCTATCAATACATAGACCGCGGTCAAAGGGAAAGAAC TTTCTATTTCAGCACCTTTAACATGGCTTTGCCCGAATTTGGGACCAGGG TGCTGTGTTTTCATCTCTCCCTGCAGGTGGTCCCCAGATGACCAGGCCGG TCCTGGGCGGAGGAGCCGGACTGTGGATCCAGTTGCTTCCCAAGACAGG CTGACAGGAGAGCAGCAAGGCCACCCCAACCGAAACCAAAGCCAGAAC GAGCAGAAAGATGCCGTCTTCCAAGTGGGGGCTGGGAGCTTCCTCCCATC CTCUGGAGCCGTGAGGTGCCCTGGAGCTGGCAGGAGCCACAGAGGACCC GGCTTTGACCGCCCCCTCTGGGACCCCACAATCAGGACCCTGACTCAGATGC TGAGGGGCCTGGACAACACCCCAGGACCCTGCTGCTTCCCCAGAACCGCT CCGTGGGGCAGGCTTTCCCTTGGGCACCGATGCACCTTGAGGGCAGAGAC GGGGCCCAATAAACGTTTCCAAACCAGTGGGTGAGGGACCCGACCGGCCC GACACGGCAGCCCGGATGCAGGGACTCCGTGCTTCGCCCAGCCTCCCTTG TGGGGACATCCGTTCTCTGATTGGGTGAGTTTCAGCCACAGAGATATTCC CAGGACTACAAAGCTGGGTCCCTTGGGGCACCTGCTGTCACAAAAAGACA AGGCCCTGACCCCAGTAGCCAAGTTCCCCCAGGGGCTCCCCAGGGTCTG TAACTTCTGTCTGCAGTTGGCTTTCAGCCTCGGGTGGGGGCAAGCCCTGC ATCTCAGGCTCCCGGGAGAACTTGCTGCCTCCACAGCAGAGCCAGGGGCC TGCTGACCACCTGCGCCGGGTCGGATCTGGTCTAGAATGCTGCTAAGGTG TTGCCAGGACTCAGGAATGAAGCCATCCCAGGTTTTGAATCCCCGGTCCC

ACTGTGCGGCCCAGCAGGGTCTGGAAGATTTCGCAGCTGGCTCGGGTCA

GGCCAGGAACCCCAAACCAAACCAGAATCA Contig 105 (1820 bp)

AGTGAGCCCTGCAGGACAGTCTGCTGAGGGGTCTCTGGGCTCCTCAGAGG CTCATGGCCACUGGCACTGGGAGGATAGCAGGTGGACCCCTGCATCCAGG TCCCAGGTCCCAGGTCCCAGACCCCCGGACAGGCTTTCTATCTGCAGGAG GGGGGCTCCTGGGGCAGCAGGGATGTGGCTGTGAGGCCTCGTCAGTCTCC CACACACACACGCACGCACGCACACACAGAGGCGTGACCAGGGCTGCA GACAGGGCCATGGGAGGACTGCCCGGCAGTGCACCCAGATGGCCACACGG TGGGGCCCTCGTCCCACTTTTGCTGCTGATGCTTCCGCCCAGCCTGCTGG GASCAAGCACTAGCTTCCCAGGGCTCTGACCAGAGAGGGGATGGGAGGGGT CATGGCTCAACAGGCGCCAGGGAATGGGAATAGGATCTGAGGGGCGGGGGCAAGGGGCCCAGGCCAGGCGAGGCTGCACTGCAGAGCTCCCTGCACCTGCAC GACCAGCCACAGGCCAACAGCTGCAGGCAGAGCAGGGCTGCTCCTGTCCC CAGAAGCTGGCACAGCACATGGGGTCTGACAGCCCCACCCCGGGCCTCCC ACAGAGGGGGGGTCCCCCAAACTCCTCCCCGTCCCACCTCACAGCTCA GCACGCACACACATGAATGCACCTGCAAGCACACACTCACACGTAAGCAG CACACACGCACACACTCAAACACGTACATGCAAGCACATGCTGGTCCT GGAGTGTTGGGGGGCTGGCTCCAACGCCCTCGCTCAACAGGCACCAACGC TGGACTGAGATAAGCCGGGGCGCTGGCTCCCTTGGGGCCGCTCAGCAGGT TTGACGCCCACACAGGTGGCACTGCCTCTTTCAGAAGACGGATGTGGCC ATGCCACCCTCACAGCCTCACCAGTCCCCCCTCAGCTTTAGTGGTGTCCC TGTCACTGTACCCGGGGCCTTCCTTCTTCCAGGGCCAAAAGCGAGTTCAG GGGACAGTGGCGCCCCCATAATTACTCACCCAGGGTGCTGTCCTCTGTGG TGGCCTTGAGGCCAAGGTGCTCCCATGGGGGCCCACAGGGCTGGCAGGGT CACTTCCTGAGAGCACCCAGGGCCAGGGCCAGGCCTGGCCGGT

Contig 106 (1500 bp)

"IGCCGAATAGAGG"GGAAACCAAGACCCGAAAAAATGTCCACATTTTTCA ATTATTAGAAATTTAGAAAAATATTTTACAGGAGTTAAAAGGTATTCCAT TCTGGGGGGGGGGGCATGCCCACGGCATGCAGGCATTCCCCGACCAGC GACTGAACTCGAGCCACGGCAGTCACCATGCTGGATCCTTAACCTGCTGA GCCCTGGGCAACTCCAGACACTCCATATTCATGTAAACTATTTTTTAAC CAAAAAAATGACAAAGCTTTTCAAAACAAAACACATTTCATGGGAAGAGT GGCATTGCTTCACGCCTGGATGGTCGCTGCGGCCTTGCCGGACGACGAGGG CCCCGCGGGAGCGCCTCCGCACGCCGCATCAGGACGTGGTGTCCAGGGA AGCGGGGTCACTTCACGGCCTCTCGGGTGCGCGTGGGTTTCCTTTTCGGC ACCACACCCGGACTCAGCACTTGGGGGTTCTTAAACGTGAGAGGCACTGC GGGGCTCGAAGCCACATCACTGACCTCCTCAGACTCTGTTATGTGAAAAC CCATCCGTCCACGAGACCAAAGAGACAGACGAACAAACGCAAGGTGGCGC CTAGGTTGGGCACAGCATGAGGGCAGAGCGGAAACCTTGGCGAAATCCCG GCGAAGCCTGGACGTCGCCAGCTCTTAÇTTGACGCAAACATAGGGGGATT CAGGAACTCTCTTTACCGCATTTGCAATTAATTTGCTGCAAATCTAAAAT CGTTCCAACCACAATGCTCACTGCATGGAAAAACCCAGGGGTAGGTCTCG CCCGATCAGGATGTTTTCCCGTGCCCTCTGTGCGGGTGCTGCCCCCTGCG CTGGTCAGTGAGAAGTGTCCCTCCACCGACGACATGAAACTTCCCAGGTC CACGCTCTCTGCTGTCCTGGACGAAAACTCATCTCTGTGAATCTCCCGCC AGCTCCGCGGGAGCCTTCCAGGGCTGGAAGGACGGCCGTCCCGTTCCAGG GGGCAGGTGCACGCTTCCCAAAGCTCCGCGTCCTGCTAGGACGCTCAGAC GGCATCACCCACAAACCCCACGAACTGTTTCCCTCGAGGCGACAGGCTCG CCCTTCTCCGAGAAAGCAGCCCGCACACGTCAGCAAGGGGCCAGCTGCGT TTGTAACTCAAATGGCCACATAGAGTTTGTCCTGGAGGCACGGGGTCTGT CTGGGCCGCACCACTGCACACGCAGAATATGCTGGGACACGCTCCGGGGT CCAGCTTCATGGAATTAATAAAGTTTACTGCTTCACCAAGTACATTCTTA AGTGTAGCTGGCCGCCAGCCTGGGCGTCCGCTCCGAGGCTGCCTCTCTGC CTGGAACCCTTGTGCTGGGGGACCCTCTCTCCAGCCCCACCCCAGCCCCG AGCCCAGGCAACATCCTTCTTGTAAGACACCCGCTACCCTGCCCTCCCGC TTCTCCTTCTCTGGATCCAATCTCCTCCGCTTCTAAGCTCTCTTGAGGCT Contig 107 (550 bp)

TAACCCACTGACCGAGGCCAGGGATCAAACCTGCAACCTCATGCTTCCTA GTCGGTTCGGTAACCACTGCGCCACAACGGGAACTCCTTTGCTTTTGTTT TTAGGATTTCACATACACGTGATAACGTGCCGTATTTATCTTTCTCATCT TCCAGGATTATCTTCTTTTTCTGTTCATCTGTGGAGGACACAGGCTGCGT CCGTGTGACGCTCTGCCGGGAATACGGGGGCCGATCGCTTTCTGAGCCAG TGTTCTCATTTTCTTGGGAGAAGTACCCGGAGTGGAACGGCTGGGTCGTC CTGCAGTTCTGTGCTGCATTTTTTGAAGACGCTCGGAGCGCTTTCCACAG TGGCTGCACCGACTGACATTCCCACCGAAGTGCACGGATTTCCCCATCCT TTTTCCACGTTTTCCCCGCACTTGCTATTTTTGCCCTGTGGATGTCGGCC TCTCCGTCAGGTGTGAGGGGGGTCTCCGTGCGGCCCAGGCGAGGAGCGAC CGTGAGCGTCGTTTCACGTTCCTGTTGGGCCACCTGCGTGGCTTCTCCGG AAAAAGGGCTGTTCAGGCTTCTTGCCCATTTCTCAGTCTGATTGTTTGGG GGGTTTGCTGTTGAGTTGTGAGTTCCGCACGTATGGGGGGCATCAACC CTTTATCAGCTATGCGATTGGCAAGTCCGTTCTCCCATGTTCCGCCGGCC GCCTTGGCACGTGTGGGCGGTCTCCTTGGCTCTTGGTGCAGAAGGC TGTTTTGATGTCAGATGCAAAAATCCATTGCCAGGGTCTGTGCCGAGAAC Contig 110 (306 bp)

CGCCACCTCAATCGCCGGTTTGTTCTGCAACACGGTCCAGATAACCAGCG CACCTAACAGGTCGAACACTGCCAGAACTGCGAACAGCGGGCTGAAGCCG ATGGTGTCAGCCAGTGCACCGACAACCAGCGCAACAGCGTACTTGCCAG CCATGCGGACATCCCGGTTAAACCGTTTGCCGTTGCCACTTCGTTACGAC CAAACACATCGGAAGAGAGCGTAATCAGCCCCCAGACAGTGCCTGGTGG GCAAAACCACCGATACACAGCAGCATAATTGCGACATACGGGTTGGTGAA CAGGCC

Contig 111 (800 bp)

TCCTGGCTCTGTAAGACCTTGAAAACACCTCATTCCTCTGGTCTTGGCCT GCTCTTCGGTACGCCAAGTTGCTGAGACTGATGTGGGGATCAGTGGGGAG CAGGAATCTTTCTGATTCAGCCGTTTCAAAGTGTCCCAAGCAGAAGCTGT GATGGCAATGCCAAGGCTATCCATGGAGGTGGCTGTGCCAGGGGCCCCAT TTCCTGGGAGCCCATTCCAGGAAAGGAATCTTGTAGCCCCAGGCTCCAGC GAGCTGTGGATGGTAAGCAGGTGGCCCAAGTCCAATTTATGTCTGTGGTC CCAGCAGGGTGCCCAGGAGGCCCCTCGTAACTCTTAAGAATCTTGGTCTG GTCAGCTAAATTGTATGACCATTGTACTGAGCACACATCCCGTTTAAGTA GAATTTTCAAGGATGACTAGGAGTTTGCCACCTGAAGGCAGGAAGGGCAT TCCAGGCAGAGGTACAGAGGTGAGAGGGAGGCTCTGACACTTTGGGCGT GCAGGGGGTTTGATGTGACTGCAGCTGGCACACAGTGTATGCCCAGGCCT GGCACGGCTGTGTTGGTGTTTGGAGAGGAAGGGAGAGGTGAGTTGAGCCC AAGGTCTTCCAGGCCAAAAGACTGAAGGTGACCGCGGCTGTCCGGGGCTG GCCCGCAGACCAGGAGGAGCAGGTGGGAGCTGGCTCTTGTTCCGGGGAC Contig 112 (3062 bp)

AGCTTTAGGCTGTTGGTCTAAAGGTCCTGCCTCCTGGAAGAGACACGCCT CTGTCAGCGGACACTGCTAAACCTAAAGGAAGAACTGCCACCTGGTCACG GGACTTCCTAGGCCAACCAACCTACAGGTGACGGCCCGGAGCATCACGAG **GAGGTAGGGGACGGGAAGGGATGCATTTGCTGCTCAGCGGATCCACTGGG** GCGTTTCTGGAGCCCCCACGCCCACACTTTACTGCAAATGCACAAGCCCC AGGCAGCAGGACAAGTCACAGTAGCTCTGGGTTATCCAAGGAGTCAGGGA CCTACCTGGAAGAGTCTAGAACAGGTGACAGAGGAGGAGGATGGTAC CAGCAGTATAGGGAGAATCAGAAATCTGACCCACCCTGGGGGCCTGACTG GGGCTGGGCCACGGGAGTTATGGGCCCCAGGTAGCATCAGAGGCTCCCAG UTACAGGCACAAGCAGCAACCACAGGAGGATCCAGGCCAGGGAGCATCC AAGAAGCAGAAGCTCCACCTTAGGTACAGTTCTGGCACCTCCAAGTT GAGAACATGTCCTAGACAGTGCCTGACCUCAACCCAATGGAGTGTCTGGG ACTAGACTAGGCACGCCATTTTGGTCCCAGGTTGCCCCATCTGTACAAAG GGTGTGCGGCCCCCAGGGGGACACAATGAGCTCCCATGGGAAGGGTCTTC CGAATCTCCTTAGAAGCAGATGTAAGAGGTGACGTCCAGCTTGTGCCTGG GATGTAGAAGTGGAAAAAGCACCCCTCCCCCGACAAGGATGAAAGCAAGA GGCACAAAACAACCTGAAATTCCCAACGCCCCTGGAGATCCTTGGAGAAC TGGGATTCTCCACCTGTAGGGGCACCTGTGAGGAGAGGCTCTGTGAGCACCTGCTGACCTGCCACAGAGGATGCCCAATACTAAGAAGCATCAGCTAAAA GTCTCCAGGAATTCCTGGAAGCTGAGGAAGGGCTCAUGAGAGGGTACAGA AGCCCTGGGGCTATAGATATAAGGGACGTGCACACCCACTTGCAGGTCCC CATGGACCCCAGGGACATTCACAGTGATGGGCAAGATTCCCAAAATGCAC CCCTTGTGTGTGGGCCTGGTTCGGTGGGTCAGCAGACACCACACCAAAGC TGAGCCTTGAGATGCTGGGGCACGTGAAAAACACTGTCACACTTAGGTCC TGCTGAAAACTGACTGCGGCCAGCGGAAAGAATCATAAAGACCCTACACC CACACACAGCCTTAATTACAGCTGTGAGTGGGGCTGGAGCCCCAAGAATG CCCCACCACCAGGCTGACAACTAACAGGTCATGTTGCAATATCACTGGGA ATGTTCTAGGAGTGTAGAAAGACACCAACTAGGGCATGATGCAAAGAT AATACTTCAGCCTGGGAGTGGATGTGACACAGGGAAAAGCATAAAGTGAT GGCAGAGGACTTTGATGTCAGTGATGGAAGCCACAAAAACTTCTAGCTTA GCTCCATTCCCAACAAGATTGACTGCAAACCCCATGCTAAAACAACAGCA AAAAGAAAGAATCCTCATTTCCAGGCATAAAATTTTTCCCCCAGTCTCTG CTGTCCTCCATAAGATGTCTGATTTCAACAGGAATTACGAGCUTATAAGA AAGGCAAGAAAAACTACACACTGTCAAGAGAAAGCCATCAGAATAACCA GACTCGTAGCACAGACACTGGAATTGTCAGGATATTTTAAATAACCGTGA CAAATACATTAAAGATTCTAATGAGAGGGGGTAGACATGTAAGATCACA TAGATTTCAGCAAAGAGATGAAACTCGAAGGAAAATTAAATGGGAGCCCT AGAGTGAAAAACACTGTAGCAGAGAAGATGGGTTCATCCGTAAACATGAC ACAGCTTAGGAAAGAATCAGTGAACTTGAAGACAGGGCCACAGAAAATAT ATAAAAGAACAAAGCATCCAAGAGCTGGAGGGTGACACTGAAGAAGAGAG CATAGGCATAGCTGGAATCTCAGAAAGAGAGAAAGAAATAACCCAAGATG TAATGGATGAGAATTTCACAGAAGCGTTGTCAAGCAACAAACCATACATC CAAGAAGCTCAGAGAACACCAAGCAAGGTAAGTACTGTAAAAAAATAGCC CGAGGTATACCTCATTCAGGCTGCTGAAAATCCATGACAAAAGAAGTCTT GAAAGTAGCCAGAAACAGAAGGCGTGTTCCATTCAGAGGGAAAAGACACC ATTGTTGCCAGAAACCAAATAAACCAGGGCTGAAAGGGTAAAACTTTTTT TTTTTTTTTTTTTTTTGGCCATGCCTGTGGCATGTGGAGGTTTCCCGA TCAGGGATCAAC

Contig 113 (1300 bp)

AGGAGTCGAATCGGAGCTGCAGCTACAAGCCTACCCCACAGCCACAGCGA
CACAGGATCTGAGCCATGTCTTGCAGCTACACCACACGCTCCCGGCAATAT
TGGATCCTTAACCCACTGAGCAAGCCACAGGACTCCCGGCAATAT
TGGATACTAGTTGGGTTTGTTACCACTGAGCACAGGGACTCACCGTGCTCAT
GGATACTAGTTGGGTTCAGAACTCTTTAATTTTTTAGTGAGGTATAGA
TTATATTTTGAAGGTTCAGAACTCTTTAATTTTTTAGTGAGGTATAGA
ATGGGTGCTGGACCTGCTGGGTGCCTTCTTCAAATGAACCACAAGCCCTC
CCTCCGCCCGTATGCAAAATTTAACTCGAGGGGCTCATAGACATAAACGT
AAACTCTAAAAGCTATAAAATTTCCAGAAGAAAACGTAAGGAAAACCTTTG
GGGTCTTGGGCAAAAGATTTCTTACCCATGACAGCAAAATTACAATCTACA
GAAGAACTGGTGGCCTTTATCGGCATTTAAAACGCCTGCCCTTTGAATGA
TGCTGTCGCAAAACCGAACATGCAGCAAAACGGATGCAACTAGCAGGTCT
CACACTCAGTGACCCACCTCAGAAAGGGAAAACGCACCTCCAAGAGA
AGAACTCATAAAACACGGCCCCCGTGAACCGCACCTCAAGAGA
AGAACTCATACAACGCACCACACTTAAAACACGGCCCCCCGTGAACCGCCCCCCTTAAGAGAG
AGACAGACCTACACACCCACCACAATTTTGGGCTTCCCGAGGGGGGATGCCGG

Contig 114 (3000 bp) TGTGAGACCCCTTGGCGGGCCAGGACCCCCCAAGGTGACCGAAGGCCTCA GCGCCCCAGCCGCCCATCCCCCTCTTTCCCGACACAGGATTTTTTTCC CACCAAGCTCTGTTCCCTTGGTCACGCTCTCACTTGAGCAGCCTCAGGGT CTCCCGGTGCCTGTATCCACGACAGCGTGACCTTCTTGGTGTGTCAACCC AGGACCCCACGCTGGCCAGCCACGCCTTCCCAGAGCACCCCCGCCCATCC TCAGAGTCCAGAGGAAAGGCCCCCATTGACCCCAGAAACCAAAACGCAGA GACTCTGGGACGCCAGCAAGAACGTACACTGACTCCCACCTGCTTCAGGC ACCCAGCCAGGGGTGGGTTATGAGCGACCCCGTGGAAGGGCCTTCTTGTC CATCGAGGGGCTTCCAGGGGGCTCCTAGACGGGGATGAGTGTGGCAACATG TCGCCGCATTACAAAAGACCCTGCAGTGCTGCTGGGATGGGTCCCCCGGC TAGAAAAGCAAAGGATTCCAGCCCAGTCGAGTAGGAGGCGUCCTCGGAGG CTGCAGAGGCGCGGGGGGCGCTGACCACCACCACCACCAGCCCCGTGTTGG AGGGGACGCCCGGCCCGGCTGCAGCCGGTGCGCCTCCGGATAAGCTCCTA AGAGGCCGCGTGCCCCATGCACGCGCGTGCACACACTCGCTGCCCGAGGG TECTTCAGCACAGACCTTGTUUGGACGGAGGACCTGGCAGGGGTGTGGCT CTGGGGAAGGGGTCTGTCCCAGGAACCCTGTTCTGGATTTGGGGGTGGGC GTGGATATCCCGTCCCAACCTACAGAAGGGGGGGGTTAAAAAAGAGCCCC TTTGGTGTGAGGGGCCAGCAATCCTTTGGCTTTTTCTTGGCCCACTTGGA GCTTGACGTCTGGTCAGTGACTGGGAGCCAGGGCCAGAGGGGGGGCAGCCG GGCTGAGCCAGGTTCAGGCCAACCATCTCTCGGCCACACTCCCGAGGTCG GGCAGCTACGGGGCCCCCAGAGACACAAGCCCCAGGGGTCCTTCCCCCCC GCCCCTGCCCAGATCACCAGGAGACCCAAGCAGCTCTGCCTCCCGTG CCTGAGAAATGCCCCATCTGGGTACCCAAATCACCCTCCCAGAAGGTAGA TCCCAGGGGCGAGGGGACTCCGTTTGGGGCACAGACGGAGGCAGAGCGGG CTGATGGATTCTCCCCCGGTTCAGGGATGCTGGCTGCCTCCAGGA GCCGGCGGTGCCATCTGATCTGATTAAGGCCTGCAGTCCCAGCTGGGCGG GCACACCCTGGGGGCTCGGCGGGGGGGAAGAAGGCGCTGTCGCCCCAGC CGGTCAGGCTCGCTTTCTCTTCATTTCCTCTCCATTAAAAGTGTCAGAAC CATTTATTGATTTTTTAAATCAGGACGTGCTGTCCGTGACACAGCAAAGT GAACAAAATCAGAGCAAAGAGAGGCCAGGGCTGAAGCCCCAGAGGGCGGC GCCTCCAATCCGGGTTGTGCCCCGGGGCTCCAAGCCCCTTCTTCTTGG GGTCCTGGGCGTAGTGGCCAGGGCAGAATGCACCTGCCGTCATCCTGGGA GGCTTGGCCATCGCTGGCTTCTGTCTCATGACGCACCGTCGTTCCATATC TATCTGCCCACCATCGGCGCTGGGGCCACGTGGAGCCCAGCCGGCTGACT TCCCGCTCGCACGCAGGCACTGATTGCAGGAACGAGGACATCCAGCCCC CGCCTCTCAATGCCCCGGGTGCTGAGAGCATTTCGCCCAAACGGCTTGGG GGCCTGCCCGTGTCTGCCCGTGGCCTCCAGCACCCTCGGCTGCCAGGCTG CTCTGGAGAGGTGCCCGGGGGCCGAGGGCCAGGGGCACCCTGTTCTGCCC CACGTCTCTCTGTCCTGCAAAGTTCCACCAGACGCGTGCTATACCCTG GGAGTCAGGAGGATGGGGGATAGTTGGGGCTTGACGTCTGTTTCTGAAAA AACACCGTTTTCCCTGAAATATATATGTATTAATTTTTCGTCAAGATAAA ACTGTGTATAGTTTTTCGTGATGAGAAAACGCATCCATCTTCCTTAGAAA GCCTGAAGAGGTACAGGAGCCTATAAAGGACAAGATGACAGATGCCTCTA ACGCACACCAAATGTGCGGTGGCCCCCAGGGGACCGCATAGACGGGGCGG CTCCAGATGGCCACCGTGTGCGAGGGACACGGTTCAGGGTGGCAGAGTAT

TCATGGAAGCCCTTATCACAACCTCGGATCCAAAACCCACTGCGCGAGTC CAGGGATAGAACTCGCATCCCCACAGACCCTATGTTGGGGTCTTAACCAG CTGAGCCACATGGAAACTGGGTAATCTATTTTTAGATGTTCCTAGGGTTT TTGGCCTTGCCTGTACGTGGGGACGCTGCTGGGCCAGGGATCAAACCCGC GCCACAGCTGTGACCCAAGCAGGAGCAGTGACAGCACCGGATCCTTAAGCA CGAGGCCAGCAGGGAGCCCCTGTGTTTAGATTTTGGTGAGGATACTGCGT GGGATTCAGGATATTCACTTTGGGGCTGTTGGAATTGCCCGTCGCTGTTT AAGCAAAGAGAAATCCCTTCACTCTGTGTAACTGTGGGGAAATCCTTTAG TCTCTTGAAACCATTGCGTGTGTTTAAGAGTGGTAACTCTGCCACCATAA ATGCCCAGACCAGCGCCTTCCTGAGATCCGCTTTTGTTGCAAATATCTGG TTTGAATGUTTTGATCCCCCGCACCAGACCAGGGTGGGCGGACGCCGCCG GGGACCCGACGTGACCATCGTGCTTCTGTATCCGCCCTTTCTCCGGCACG CGCCCCTGGTTGCCTCTGGCTGCTTTTAGTGGAGGAACTGAAGCCTCGC CACCCAGACCCCGAGACCGCAGGACCCACAATGCTTCAAACACCTGCCCT CTGACTTTTACAGGTCAAGTTCGCCAACGCCGAATTTGCACCGATTGGCT ACAGAGAGCACGGTGGCGCCAAGCCTCCACTTGGAGTTTTATAAGGTCTC CCTCCAGCTCGCAATGAAAATGAGCTGTGATAAGGCAAAGACAAAATTAG TATGAAATCCAGATGCTTCATCTACAATACAATGACCGCGGGATTTGGGT CTGAGCGACTGAAATCAAGGTGGGCTTCCGGAGGGAGGCTGTTAGAGGAA AGGCATTCACGCAGGCTCAGGTCCGAGAGGCTTCCACACCCCTAAGAGGG CTGAGACGGCAAGTAGGGACCAAGCCCCGCAGTCGGGAGAGCTGGGCAGG AAGGAAGTCTGAGGTCACCCCCACCTGGGGAGGAACTGCCTAGAGAAGCG GGGGCGGAAGCAGGGGATGCCCAGTCCCAAGACAGGGACAGGGCGGAAA GGGCTCTCTGCAGGCCCTCAATGCTGCCACAGTGTCCTCGTAAGAGGGAG GCAGAGAGAATTGACACCGGGGAGACCACGGGACCACGGAGGTGGAGACC GGGCTGCCGCGCGTGCCAGTTGCTCCCGAAGCCGGCCCCTCCCCCAGAG CCTTTGGGAAGAGGCGCCAACCTGCAGTTCTGCTACTCGGGGACAGGGAC AGGGACAGCCCCTGGAGCCGCCTCTTAGGGGCAGCATCCCCCAGAACCT TCCTTAACAGACCATCTGGAGAGAGATGGGTCTGGGCTGCAGCTCCTGGA ACTGTTTTGCCCACCCGGCGAGCACCAGTGGGTGCCAGCCTGGGCTGCCC AGCCTCAGGGCCGGGGAGGGCTGAGGGCACTGGGGCCCGGCTCTGGGACT CCCCTGCCTCCTGCCCGTGCAGGACAGCCACCTCCCAGCATCTGCTTCCT GCCACCCACACTCCCCAGGACCGTCAGCCCAGGCATGCCCTGGCGTCGGC CACTCACACCACAGGCCAGGAACCCAAGGGGGCAACACAGAAGGGCAGTT GCCATCTGCAGATCCGAATGGACAAACTGGGGTCCGTGATGATGGCAGGCT CTGGGCGCCCGGGCTGGCAGGGGGGGCCAGGACTGTGCGGCCATCACAGGA AGGGCATGACGGGGTGAAAGCAAGAGTGGAAACCTCTGCCACCCGCCTGG 

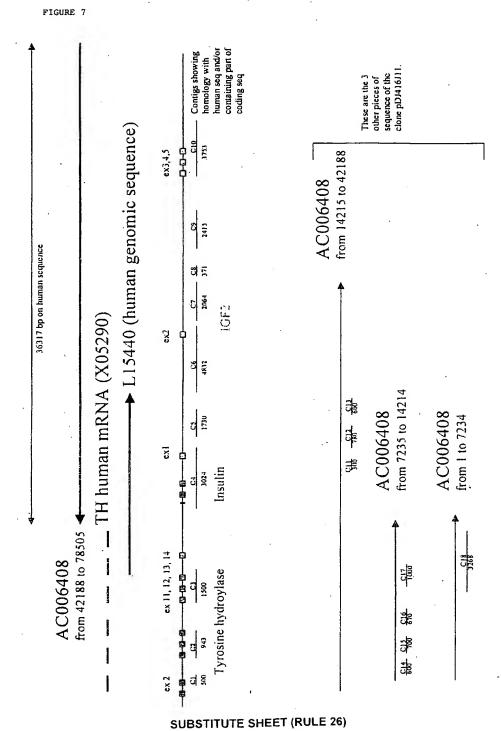


FIGURE 8

#### Contig 1 (1040 bp)

GCGCGCCGGATCCTTAATTAAGTCTUAGAGATCTGCGGCCGGCCCAGGGTCTGCTTCTG GCCAAGTGTGGGGCTCTGCTCCATCCTGGCTCGGAGGTCCACCCATGCCAAAGCCTGGGG TCCTCCCACTGAATATTTGGGGGTCCACTCGTGCCAAAGGCTGGGTGTCCAGTGTGCCAA CGGTACATGGAAGCAATGTCTTCCCAAGGACCGTCCAAGGTGTGGTCAGGCCTGGACAGC TGTGAGTCCCTTCGGGACTAGACTTGGTGGCCGAACCCTAGGGACCGTGCCCGAGGGCCC CCACGAGGCCAGGTGTTTGCCCCCAGGGACAGAACGGCCAAGGGTGGCCGAGGGTTCTTTT CAGGGCTGAGAGAGCCCGGCTTGTCACTAGGGCGCCCCGGTGAGCCCAGCCGGCATGCCG TGTCCAGACGTTGGATGGGGCAGCGAGGGGACTGGGGTGCUCCAGCCCCGTGGGAAGCC CGCCCTGTGGAAGCCGCTGTGCTCGCCACAACAAGCACCGTCGACTAGCTGGTGAATCAG CGCCCGTCGCCCGCGTAATCCCAGGCGCTTTCTGCCCAACCTGAGCCCTGACCCCACACC CCTTGCGACCGCTCCGTGGACCCTGGGGCGATGAGGTGAACCGTGGGCTTGGCCATCGTG CCACCCAGTGGGGGCTGGGCAGGGAGCCGCCTCCACCTCCGCCCTGAGGGGACGGGACTC GGGGGAGAAGGCCCTCTTTGGAGAATTCCAGGACGGGTGAGGAACGTCTGCTGGACCGGC CGGGTCGGACCTGGGCCTTG

#### Contig 2 (9234 bp)

GGCAACCAGGGGAAGATGGGGAAGCGGGGTGCAGGGCGTTTGCGCGGGCCAAGGACCAC CTTGGAAATCTGGAGCCTGGCAGGAGCGCCGCAGGGTTGAGGGGCCTGGCTTGGGCAGGGC TGGCTGGCACCTGGGGGGCTGGCGGGTTGAGGTCCGGGCTCCCAGGTGCCCTATAGGCA GGGCAACATCGGCATGGGGGTGACAGGCCCGAGCTGGGGTGCGGAAGAGGGGGGGA GCCAGGCATTCATCCCGGTCAATTTTGGTTTCAGGTCGTGGCGGCTGGTGGTCAGGGGGA GTTGGAGAGAGGTTCGCCCCGGGGCCTGGGGGCAGCTGTAGCTGGCAGCTGTGGGC TCTGGCCTCCTGCATCCGGAGGTTCTGGGGAGCGAGGGGCGGCGAGGCGAAGCGGCTGAC CCCCCGGCAGAGTGGCGGCGGACGACAGGCAGAAGGCGGCAGAACAGGTGACACGTCTCAG GGGGAGCTGGGACCGGGGGGGCCTGGGGGGCCGTCCCAGGTGGAAAGAGCATCT CAAGCGAGTCTGGTGGGAGACGAGGCAGGGAGGCGAGACGCGAGACGCGG GGGGCATTCCAGGCCCGGGTCGGACAGGACCCGTCGGGGGTGTCAGGACAGTGGGGTCCC CAGCCGCCACTTCACCCACTGCAATTCATTTAGTAGCAGGTACAGGAGCGGCTCTGGCCG GGCCTCTTGAGGCCTGAGCCTCGAGGGCCGGAGAATGGGAAAGAAGGTGCAGTG TGCCAGACAGACGTCACCTGGAGGGAGCACGGCCGTGGGGACGGGCCCCAGAGAGATTTC GGCAGCAGGGGGGCGGGGCCCAGCCTGCGGACGTGCGTTCCCACGCAGCACTGCGG CCCAGGGGCTGGCGCGCAGGGCCCCCGGTGTCCTTGGTGGCACTGTGCGCCCTCGCCGC TCGCCCCTGGGACTGGCACGCAGACAGGACACCCCAGGGGAGTCAAGGCCACTGACG GGGGACGCAGGCAGGGCGAGGCGCGAGCCTGGCGGCGAGGCCAAGGCGGGCCT CTGCGGCTGACAACTGAGCACATATGGGTACCTTTGCGCTCGCACCGGAGACAGGTGAGT GTCTGGCCCGGCCTGCCGCCCTCCCGGCCCCCCACTGCCTCTGCCCTCCCCCTCGACC AGGGCCCTCTGCTTCCCCACAGCCTCGTCTCCAGTGGGGGTGGACACACTGCCAGCACCA CAGGCCGGACGCCAGGATGTGCTTGGAGGGACATGACACTCCGGTGTGACGGAGAGGG ACAGACGTGACGCCGTCCGGCCTTCCTGGTGAGCGCAGGTCCAGGCCTTGGCCCCCAGGC CAGCCGCCCCACCCCTCATGGCCGTCTTCTGTCCCGCAGAACACTCTCGGCTG GCCCCGCGGGGGAGCTGCCACACCCAGCGTCTGTTCCTTTGCCTTCCTGAAGGAGCACGT GCATGACTGCTCTCTGGACCCCAGAACCCTCAAACGACAAGGTGAGGCAGGTCCCGC CTCGCCCCACACGTGGAAGGGGCGTGGGCGAGAGCCGGGCGCTCACGGTGCCCCCCTCCC CCTGCAGAGATGGTGCTACCCAGCTCATGCCTGGGCCTTGGACCCGGACTTCTTCAAGTC CTCCTAGCTCTGACTCAAGAATATGCTGCATTCTGGAGCCACTACACTACTTGACTCAGG

AATCAGETCTGGAAGGTGGGCGCGCGCTCCTCCCGC'fCCCGGAGCCCCGCCGCCGCTGCCCG CTCCCGCTCACGTCCTGTCTCTCTCGTCCGCAGGTTGAGCCAAAGGAACAGACGTC CCTTTTTTTCCTCTGTCTTTTCTCTTCTTTCTTTCCTCTCTTTGCTCAGAAGACTCGG TGCTTTGGAGTCCTGGAACCAGCCCCGGGTCTCGGAGCGGGTGTGTGAGCTGCCGAGTGG GACTGGGTGGGGCCTGAGTGTGTGGGGCCCCGCCCTCCCCTCTCCTAGTCTGGAAGCTC CGACCACCGAGCAGACCTCAAACGCTGCACTGAGTGTCCATCTCGTCATGTGCCCCTCCT CGCCAGGGCCACCCCAGAGCCCTGGACTCATCAATAAACTCAGTTACCGGAATCTGTCTC AGGGGCTTTGCAATTGGGCTGGGGGTGCGCCGGCGAAGGGGGGGATGAGATGGGGAACAT GCAAGGAAGGCCCTGTGGGCTGGGGGACACAGAATGGGTGCGGAGGGGGGCTCACAGGACT CGGGGGGTAATGAACGTGGGGCTGGGCGCAAAGGGGAGTGGGAACGTGGGGATCAGGGCGG GGGGCCTGGAGGATGCAGGGTCCCTGCAGGGAAAGGGGGCCGAGGGCGTGAGGCATGTCC TCAGCCCTGAGAGGCCCTACCCCACAAAGCACAGCCTGCGCGCGACCTCCAGGCCCCCAA CATGCCACCCAGGCTGGCCACACCACTGGGACGCCCATGGGCGGCCACTTTCATCAAGAG CCTGCCAGGCCCTGAGTGCTGGGCTGGAGGGCACAGAGGGTCCCCCTCCCCTCACGCTTT GCGGTGCTGGGGCACCGCAGGAGTGCCCAACAGGAGACCCCAGGAAGTCTGCTGGGCTGC AGUGAAGGGCAGGTAGGGGGGCGGCCCACAGGGGCCCAGCTCAGTAGGCAGGTGGCACT GGGAGGCGGCAGAAAGTTGGAAAGGGTGGACTGGGCACGTCACCATCTCGTGGCGGCAGC CCCGGAGCCACGGCCTTGGGTGCACTGCAGCCCCCACGGTTGGTGTCCCGGTCCCAGGCA CCTCCCAGCCCTGGGCGCCTGGCGTGACGCTGGGAACGCGAGGGGAGCAGGCCTCGGAAA CAGGGCTGGGTCCTTGACCCCTTCCTCTGCTCAGGGCAGTCAGGAAATGCCTAGGGGGCC GACTGACCGAGAGGAGATAGCGGAGGCCTGGGAGACCCCGCGCTCGTGCCGTTCCCAGCG TCCGGCCCCTGGCCTTGGCTGGCCTGGTTTGGGCCCCATGAGCTCACCCCCGCCCCC CACAGCCTCCCCCCGTCTGGTCTCCTCTGGGCCCTGCTGTCCCTGACGGUGGACA GAGCCCTCCAGGGCCCCGGGGGGACGCTCCCGGTCAGCAGGGCGGGTGGGCAGCACAGC TGCGTTTGGTGAAGCCCCTGCCCAAAGCACCCTCAGCGTTTCCTCTGCGCGTCCGGCCGC CCCCGGAGGCTTTCCCAAGTCCACGGGCAACTCGCAGGCGAGCCCACTCCACCTCCATCA CGCGGGTTTGGCCAGCGGCAGAAGCACTCGCCCTTCAGGCGTCAGGAGTTAAGCCCCTCC AAGGCCCGGTGCTAATCAGCTGCCTCTCCTGGAGCTTCGCAAAGCGGGCTCTCAGAGCCC AGCTTCCCGGGGGCTCACCGTGGTGGCATGGGCACCACAGGTGGCCGGAGGGGCACCGAG CACGACGGGGCTGTGGGGGGTGGAGGAGGGAGGTTCUTGACTCCGAACCTCTACTGAGGC ACACAGAGGACACGCCCGCTTCCAGGGGAGTCAGCCTGCGAAGGGCAGAGGGGCTGTAGC CTCCCGGTCACGCCCTCGCCTCTGCCCTGGATTCCTCCTGGGGGCCCGCGGCTCGTCGGG CAGGTGAGTGCCCCTGGATGGGCGTAGGCTGGGGGGGGCGCAGGGAGCCCCGAGG CCCTGGGCCCACAGCCCTGTCTTGCCCCACACACAGGGCTGTCTACACTGGGTGCCCACT TGCTCTGCTTCTAGGCTGTTCCCTGGGCAGCTGCCTGGAGGGCCGTGGGCACAGTGCGGG CAGCCAGTGGGGAGGCCGGGGATGGGGCCGGGGATAGGGACCCCTGCCCCTGGGTGAGCC CCACCTGGGCTGGGAAGACACCAGCAGCGCCCCTTCAGGTCCATGGACCAGGGGACCCAG GGTGGACTGTGTTTACCTTCAGCCCAGGCCAGTTTCCTGCTTGAGAAAGCCCGGGAGGGG GTGCGGGACAGGCCCGGGCCCCCACGCAAAGGCAGTTTCGCAATGTCCCTGCGCTGACT GAAATGTCACCAGGCACACGCCTTGAATTTCTCCCCCAGACCTGGCAGGGGCGGGGGTGG GGGCACCGGGCTGCTGGGATCTTGGCCCCTGAACCTCCCCGGCCCTGCGGCCAGGGAGG GTTTAGGCTGAGTGACAGCCCACGGAAACCTGGACCCGACATGTCTGTGTGCCATGTGT GTCTGTGTGTGCGTCCACCTATGCGTCTGCGTGTGTCCATGTGTGTCCACATATCTGT UTCCACUTGTCTGTGTCCACGTGTCTGTGTCCACGTGTGTCCACGTGTGTCCATGTGT CTATGAGTCCTTGTGTGCATCTGTGTGCCCGTGTGTCTCTCTGTCCCCTGCAGTCC CCGTGGACCTGTCTCTTATACACATCTCAACCTG GCAGCGCCCTTCAGGTCCATGGACCAGGGGACCUAGGGTGGACTGTGTTTACCTTCAGC

FIGURE 8. CONTD.

AAGAGCAACGTCTGAGCTAGCTCCACGCGTGGGTCCATCTCGGCCCAGGTTTAATGAGCC ACTTTCAGGCAGGGATTGCACAGGAGGCAGGGTGGGAAGTGGCTCTGCTCAGACCCCTGA ACAGGGTCTGGAGATTCTCCAAGGGCACAAAAGAACGGACGATGCCCCTGGGGTCAGCGA CCCGTGTCTTGTCTCAACAAGCCCCTGACTTGGAGGCCCCAGGGTGACCCCTTAAAGGGG GAACAGAAGGTTCTAGAAGGAGCGTGGCCAGCTTTGGCTTCCCTAGGGCTGTGGTGACCA ACCAGCGGGGCCCCTTCCTGGAAGCCCACCTGCAGGCCGGCTTGCTGGGAAGGGGCCTGC TCCTCGCCGGCCCCACCCGGCGGGGCCGTTTCCTGGAAGCGGTCACTGGATATTTTGTT CCTTGTCAGCGCCGAGCTTGCATAAAGCAGACACTGAUCTCCTTGTCCTCCGGGAGCACG CGCTCCATCACCGAACACCTGGCCGGACACAGGCGGGCAGCCGGGCCTGGGGGAGCAGCG CGGGCCTGGGGCCGGACCAGCAAACGATCACGGCGCGGGGCGCGGGGCCGCGCGCTTC TGCAGGCCGCCCCACGTGCCCAGGCCCAGCGGTGCCCATCCTGCAGGCTGGGAGGACGC TGTGGGCGCAGAGCTGAGAAGGGGGCAGAGGCACTGGGGGGGCACAGCCGTGTTCCCACA CTTTGCAGAAACCTTGGCCGGCCTGGATGTCTTGCTGGGAGAGCTGGGGGAGGGGACAGG GCAGGAAGCCGGTCCCCCGAGCGGGGTAGGAAGAGGCCTCGGCCCTGGGAGGAGGAGGA ACAGGGACGTGACCTGGGGGCCGGTCCCGGGCCCAGGCGGGCTGGGAGGGCGCCTGGTGG GTCAGCGCCACTCAGAGCCCTGGCAGCAGGGGGGCCTGGGGCACGGCTGCAGGACAGAGCTC AGGACACAGATGGGGGCGAGGACTGAGTGGGCCACCACAGATGCTCCCAGGAGGTGGCCA AGGAGTGGCCTTGGGATCCCAGGATGGCCCTGGTCCCAGAAGATGCGGCAGCCCAAGGGA CCAGGCCAGGGCCACAGGGCCACAATCTGAGCAGGGCTCAGGCCCAGGGCAGAGGCCC CCTCCCACCCAGCCCTCCCTGGGCCCGCCTCTCC

GTGCAGGCAGTGGGCTCAGATGGGGCAGACATGAGACCAGGTCCAGGGAGAAGCGCCCCC TCAGGAGCACACAGACCCCCACCACGGGCTCCCCCAGG"TGGGCGGTGACATCAGCCCTG TGTCAACAGCAGGAGCTGGCAGCTCCCCACCGGGGCTTAGGGAGCGGGGACCCTGAGCCA CCCTGCCACCGCCCACCCCACCGTGGCCCACACGAGGGCCCGCTGCTCTGGGTCTGGGG CCAAGGCCCCCCAGGCGCCTGGCACTGTCTGCCCCTCCCGCTGGCTCTCCGTCTCCAGTG CAAAGCGGACCCCAGGGAGTCCCGCGGAATGTGGGACAGCCCCCCGTAGATCTCGGGGG GGCCAAGCTCTGGTTGACCTCCATCCTGGGGCTGTGGGCCTTTGGTCAGTGGGGAGGGTC ATGACACCCAGCCACCAGCTGGTGACAGCCCTGGACGTGCCGGCTCAGGGCTGGCCTGC TGAGAGACGAGAGCCTCTCTTCCCAGAACTTCTGCCTGCGATGAGGACCCAGCAGGGGCC TCTCCTCACCAGAGGGCCTCTGCCGGCTGCAGGGCCCCAGAGGCCCCAGAGGCTGGAGG CCGGGCCTTGGGAAGGCCGGACTTCCAGAAACCAGCTGCCCGCTCCGCAGCACCCCAGC CAATAAAGTTTGTCCCTGCTGGTTACTGTCCGTGTCTGAGAGGTTTCTGGAGCCTGGCCA CAATGGGCGTCAGGATGCGGCTGGGAGGGAGCCTCGCCAGTCAGAGTGTGCTGGTCTCGG GGGACCAAGGAGGGGACAGCCCCCGGTCACCAGGAGGGTCCTGTCCCCTCTCACCCCCCGG GACAGGTGAGCTCCCCGGAGCCGCCCTTCTGGGACAGGACCCCACGGCCAGGCCACGGCC CCCCCACCCGTGGTCCCTCCGTCCACGGCCGGCCTGGGGGGCCACGGGCCCAGGGCC CCCGCTCCCGTTGGCCCTCCGAGGGTGAACGACCTCGCCTGGGACGTGGGCCAGAGGGC AGGCGCCAAGAGTGACCCCCTGGGACACGTGGCTGTTTGCAGTTCTGGAGGCAGCCGAGA AGCCCTGCCGCCGCCGCCGCCGCGCGCAGGCACCGTGGGACCCGGCCTGGTGCCCCT CCCCGCCCTGCTCAGGGGCCAGCCCTCTCTGGTTCCCAGGACGCCCCGCCCCGCAGG CGGCCAGAGAGTCCCAGAGTGTTACCCTCCCACGTGTGGGATCCTGTCATATGCGACAGC GGCTGGAAGCCTAAGGCGGTGGGGCGTGGGGGTGAGAGGCCCGCAGACAACAGGAGGAGG CTGGGACACTTCAAGGGTTGACATGCTATGCCTGTCACGGATAAATGC

Contig 3 (5347 bp)

AGATGTGTATAAGAGACAGGGGCTGGGTGGGAAAGGACAGAGGGTGGGGCCGGAGGAAATG

FIGURE 8, CONTO.

GGATGCAGAGCCCACCGTGCACGCTCTGCTGGCCTTTGAGCCTCGCTGAGTCGCAAGAAG GGCATGGCTGGAGGGCCCGAGAAGCCACCCAGGCTTCCCGTGCCGAGCTGGGTGCTGGGC CCAGCCGAGCGGGCTTGACGCCACGCTTAGCCCTCCCCAGGGAGCCCAGGGTCGGAAGGA AGAGGCCGGCCGGAGGGCCGTGGCCGCTCAGGCTGGAGGGGGCCCCCGGGTCAGGATGGG CCCCAGACGTCCCCGCTCCCGGCCATCCGTCACGGAGCTGTCACCCAGGAACGTGCTCC AGACGTGCTTTCCTGCCGCCGAGGCCCCGAGCAGGCTCCAGGCGCCCCCACCCCGAACG CCCACGCACACCCTCGGTCTGCGAACACCCTGCCGTCATCCGGTGGCCCCGGTTCCCGCC GCCCGCGCCATCCGGGTGCCCCTTCCTCCCTGGGTCGGGGGCCATGCCCTCAGCGGGCAC GCAGGCCTGTGCAGGTCTGTTCTGACTCTTCCCCAAAGACGCAGGCCGGCTGCGGGCGCC CCGACCTCGTCTGAGGCCCGTTTGTGCTCACTGGCTGTCTCAGAAAGGGGTGCCCACGGG AAGCGCGTGTTCCTTGGGCCGCAAGGCAAGGGAGCCCACCCCAAGGTGGCTGAGGGCAAA GCCCTGGCTCTGCTCCCCGGGCAGGTGAGCCCACGGCAGGGGGGCTCCCCAGCAGCCTTG GCAGGAACCAGTGAGGAAGGGGTGAGGATGAAGGCAAGGGGGCCTGCGGGGACTTGGGCA AAGCCCCTGAAGAACTGAGTTCCTCGGAAAGGCCGGAGCCCTCAGCCGAGCCTCGGCCTC CGAGCGATGGAGGCGGCCCACCTGCGGCCCCAGGGTGCAGCTGTGCATCCGTCCCCCTCG GGCCTCCCCCTGCCCCCCGGCCACACTCTCCCCCTTTTGCCTTTGATCACTTGAGT GCGACAGCTTGTGCGGCCTGAGCCCCAGAGACCGCTGCCCCCTGCCCCCAGCCCCACGG GAGCGTCCACCTGGGCCTGGGCACTCATCCCTCCCGGATGAGGCCTTTCTAGCCT GGGCCGCCCGGGAGCGCAGACCCAGCCCCTCGCCCCCCTCCCCCAGTGAAGGTGCTGC CTGGTGGTCTGGGAAGCCCCTGGAACAGGGGGCGCAGGTCCCACACGGGTGCTCTGGCC TCCAGCTGCCAGGGGGGCCGCGCTCAGGCCAGGGTCCCCTCCACCAGAACCGCCAGGGC CCTGGGGAAAACCTGTCTGTGCTAACAGGGCCGCTCCCCGGGACTCCACGGAGAGGTGCG AGCGGAGGTGCCCGAAGGCGGAAGAGCCCACCCTCCACTCGGGGACCTATTTCAGCAAGA AGACGGATGGGACTGCCGGGCATGGACAAAGGAACAGGATGAACCTTCTGGAACGCACAA GGCTTCCACGGCTGACCGGTCATAGGAAGGCGCGTCTCTAGGCCAATCCACCGTCCACCG TCCATTCCCCAGCCCTCGAGAGGGGGCACGATGGACCGCTGCAGCGTGAGAGAGCTCTGG GGCGCTCCCACAGGGCAAAGTCCCAGGGCACTGACCTCAGAGCCCAACCAGGCCACCGGG GCTGGGCCCACCAGGGAGCCGGGGCCAGGGTCAGGGTCAGGGCCCAGAGTGCGGGAAAGG GTGGCCTGTTGCTTGGGCGGGGGGGCGCGCAGACGGCCCCTCGCACCCCCGGACAGCCCT GGAGCTGAGTGAAGCCCGCGGGTCACCTTGGCTGGGGTTGGGGTCTCCTGCGACCGGCAC CCCAGCTCAGGTCATCCTTGCTGTACCGCAGAGGGGCAGGGGTTCTGAGCAGGGACAGGG TGGGCCGCGCAGGAAGCCCCCTTCTCTCTGAGGCTGCCCCGGCCCTGGAGCCTCTCTGGG GCATGCCACCCCTCTCACAGACGCCTCCCAGGAGCCCCCACTTTCCTGCTGCGTGGTGAG TTGGAGCAGGTGCAGGGCATCACCACACAGCAGCAGAGGCTGTGGGGGCCCCCTGAGAGGC GCTCCCAGGTACCCTCCTCAGGGGGCTGAGCCCGGGGTTGACCCGGGACCTCGCCTGCCC CAAAGCCGGCGCCCTCCTCCCGCCCGCCCGACCAGGGCCAGAGAAGCAGGTGTGGGGCGG CACAAACCCAAGTCAGCTTCCAGATCCTGCTGGGGCCGCGTTGAAACTCGAAGCCCCCAG GCTGGGAGGTCTAGACACCCCTGCCCAGACCGACAGCCTGGGCCTGGCTCACAGCTGCCT GGGGCCCAGGGGTGCACCTGCCCTGTGGGTGGGGGTCAGAGGGCAGGGAACCCTCGGGA AGGTCCCCCAGGGTCAAGGTTGGGCCTAAGCTCCGGTGACCTCTGGGAAGTCTGGGGCTG GGTTTTETTCCCAGAGGAGAGAGGGCCACTAGCCTCAGAGGGGCTGTGGCACGGTGGGAA GGCCCCAGGTGACCCCAGAGCGTGCGAAGCCAAGCCCCCTTGACTGCAAAGC GCAAAGGGCAGAGGTGGGGTGGGAGCCTCGACCCCCGAGCCCAGGTACACAGGGGGAAG GGCGAGGGATCCGGCAGGGGCCACACCCGCCACCCGGCAGCCCACAAAGCCTTTGGGC CCGGAGCCCAGATGGGCCCAGCCCAGCTCTGGGAACAGTCTTCCCAGAATTCCCCAGCT GGGATCTCCTAAGTGGCAAGGCCTGTTGGGAGGGGCTGGTGAGAGGCCACTCTGGCGGGA AGACCCCAGCCACCTGGAGCCCCTAGCCACTGCCTGCGGGCTCCCTAGGGATCCAGG GCCATCAGAGAAGCTCCAGCGACACTGTTTATTTTCAAATGACACTTTTTAAGAAAAACA AGGCTGTCAGGGCACGGAACGTGTCTCTGGGCCCTGTCCTCAATTCCCGGTGCCCAGTGG CCCCAACTTCCCAGCAGACCCAGCAGGGCCCCAGCTTGTCTTGGCCTGGCCGCTGGTCCT GTCACCCCAGGCCTGGAGTTCTGGAAGATTCTGCTCCTGCTCCCGTGTGCACATACCACT GCAGCCCGCCTGATCTTCCAGGTCCTCCTCCGAGCCCCCGCCTCCAGGAAGCCCTCCAGG AGAGCTCAGGAGGGTCGGCTCCCTGCGCGCAGCTGTCAGACCCCTGGGCCCACCCCGCCG GCTGCTAGGGTCCAGGTTCCCCACAAGCCCTCGGGCAGAGGCTGGGCCGCTGGGTCCCTC

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GGAGACAACTGGCTCCGAGGCCTTGCCCTAGACGGGTTTCCGGGAGCCCGTCCCCAGCGG

FIGURE 8. CONTD.

CACCCACTGAGTTTTGAACACTTGGCGCCACCCCCACACCCCAGGCGGTGGCCAGGAGGC CTCCTGGGCAGCAGACAGTCCGTGAGGTGGCCCTGGGGTGGCTCCTGACCTGGGCGCTGG CCCAGCCCTGGGCACAGCTTTCCAGATCTTGCCTGCCGCTTCCTCCAGGCTGCCTCGGCC CCTCCCGCCTGGGGGTGCCCAGCTTTTCCTGGAGGATGCCCACCCTTGCCCATGGTCAGG GAGGGGCTGAGAAACCCCACCTCCTGCCTCTGCCCGGCCTATGCCAGGGGAACCAGGTTC CCTCCCGCAGGAGGGGACCGAGTCCCTGACAGCCCACTGCAGAGGGGAGGAGGTGCCTGG CTCTGCCCCCAGCCCCACCAACCCCGTGGCTTCCTGTTTCGCAGCCCACAAAGCACTAAA GGCCGCAGGTCCTGGAACATCAAAGACCCGGGAAGTCCATTGTATTGAATTGAGTGTAAA TGAGCCTGAGGCCTGTGGCTTGCGTTTCCCACAATTACCGCTGCCCGGGAAGGGCTCCGG AACCGACACAGCCCCCAGGGCCCCTTGCCCATGTGGGGAGCCCAGGCTGGCCTGAAGAAG CCCCATAAGGTGGACCCCACTTTGAGCCCCCACGAGAGTGGGCCAAGGACCAGGTCAGGG GCTGCCCAGGCTCTGGGCCTCCTCTGCCTGCCAGGTGGGCTCCCTCGGGGCCCAGCCTGG CCTGCAGGACCTTCCCACGCTGAGTTCCCCAGCCTGGTATGAGCGTAGTGGACGGCAGCC ATGCCCAGCACTCAGGGGCCTGAGGGACAGAGCGGGAACTCCAGCCCCCGGGTCCTCGGC CCCTAGGATCCTTCTAGGTGGGGAAGCCCAAGGGAGCAGAGGGGTGAACGCAGCTGTCTG GGGCCCCAGGCTGCCGAGCAGACCCCTCCTGCTCCACTCCTCGGCCGAGTGGGCGCCGAG ATGCCGGGGCAGTGCCATTTCCCAGGCGCCACCGGAGGCTCCCAGAGGGAGTGAGGCACG AGCTCGGAGGGAGGCCGGGGGGCTGGGGAGGCAGAGAGGCCGGAGGCCGGTGAG GAGGCCCGGAGGGGCCTGGAGTCAATGACCCAGGGATTATCGTGCTGGGTCTTTGCAAA GTTGGCTGAGCAAACGCCGGAGCCAAGGGTCAGGGGGGAGACGGGACTGGCGGGGCCCCGCGG CCCCCTTTCCCCTTTCTGGAAAAAGCCTGTTTCCCAGGTCAAAATCCAGCTCATGATCCG CCCCTTTGGGACTGATGTTCAGAGGCCCAGTGGTCCCAGCACCTCTGTCCACCGCCCCC CCCACGCTCCCGGGGCCGCCAACCCCTGTGGGCTGCGAGGTGCGGGCACCTCTCCCTTCG AAGCAAAGCCCTGCCTGCGTGGGCAGCGTGATTTCCTGCTTCTCTGGGGCTGCACTTTG ACTGGGGTGGGGGGTGG

#### Contig 4 (1592 bp)

AGCCC:TCAGCCCCTCCGAGCAGCTGCTGGGCTCAGCGGGCTCGCCCCCGATGTGCGGC CGCCCTCGGCCCCCTTCCCCGAGGGCACCCCCACGGAGGCCCAGACCGGAGGCACTC GGGGCCCAGAGCCCAGGGCAAGAGTGAAGGCAGCGCCGGTGGGAGCGGCGGTCAGCGGGG-TCCAGGCTCAGTCCCAAGGAGCCCCATGCCCTGAGCCCCACTGAGCCCTGTGCAGCC CCAGCCTCCCTCCTAATCCCCCGCATTTTCCGAATTCTCGGGCCACTGCTGCTTC CTCCTCAAATTCCTGGCCCCCTCGCCCCATCCCCGCCATGGGAAAGGGCCGCGATGCCA GAGGTGCGGGGCTGCCAGGGAGAAGGGCCCAGATTAGGGGGCGTCATGGGAAAGCTGGGA GGGAACGCTACCCAGAGCCCCTCCTGCCGCAGCCTGTGCTGCTCCCTCTCCGCATTTCTG GCCTCTGAGTGCTCCCTGGAGGGAAGGGACCACTGTGTCCTGCCGGCCTCTGGCTCTGCC AGGAATGTCCATCTGTCCGGGCCGGGTTACCTGGCTCAGAGCGTGGGTACCAGCTCATCC AGCCCTGACGCCTGCTCTCGGGAACAGTGGATGGGCCAGGCGCCCCGGTCACACCCCGCA GCTGGGCTCCACAGACGGGCCCGGGATGGCCACGGAGGTGGGGGGGCGCCCCAGGGCGAG CAGGTGCAGCCCGGGGCAGGTGCTGGTGGGGGCTGTGACCCACGTGTGCAGCTCAAGGGT CCAGGAGCCCAGGGACAGAGCCTCAGGGACAGCCTCAGAGCCACAGCAGGAAGCCTG ACCCCGGGCCTCCTCTCGCACGATTCCCAGGCCAGCCTGGTCTCAGGCAGTCCAAGGTTGCACAATGGTCTCCATCGTCCACAGTTGCAGAGCCAGCACTCTCCCACTGGACGGCGGCCC TTGACCCTGTCTCTTATACACATCTCAACCCTG

#### Contig 5 (831 bp)

#### 44/48

FIGURE 8. CONTD.

#### Contig 6 (4634 bp)

CTCTGGGCTAGCACCGTGGGGGCTTTGCCAGAGTGGAACTGAACTGGGTCCACCCGGAG CCCAGAGGGCGTGAATGGGAGGCAGAGCCCATCCTGGGAATGGACCAGAAGAAAGGGAG CACCACTCCCGAAGCTGATCTGGAGCACACGCGTCGTTAAAGCCGCCATCGAGGCCCCA CTTCTGACAGACGGAAGGGGGCAGAGTGCCTTCCTCACCGGCCTCGCCCTGGGAAGGCCC CTCCCTGCAGCCCAGGAAGCCAGCAGCAGGTGACAGAGCCAGGGGCCCAGGGCCCCAGGG ACGGGCTCGCGCGCCGAGCCGGGGGTCCCTTGGCGTCCCCATCCTCTCGTCCTCGAGCC TGGCGTCATCGTCTCCGCGTCTCTACCCACTGAGCAAAGACACACGAAATGAAGCTCGAA CGAGCACAGCCAAAGAACGGCCGTTTCTGTCCTTTCTTCTTAATCCCTTTGGCTTAGGGT GTGACCANTCCCAGGCCACCCAGGCTGTGCCCTGCGTCGTGGGCCATTTCCCAGCCGGCC AGAGATGGAGCAGCCACTGCGGGTCCCCGAGTCTCGGTGAGACAGTCAAGGATGGACCTT GGATGGAGACCGGCGTGCGGCCATGTCCGTGGGTGAAGGAGGCGTGCAGGCCGTGCTCGG GGACATGGTTGCTGTCCCCTCGGCCAAACCATGAAAAGCAGCCCTCTCCCCCAACCCCCA GCACCAACCCGGAGACCACCCTCGGCCGGAGCCCAGCACGGCCACCGTCACGTCTCGGTC GTCCAGCTTGGGACAGGTCAGTTCCCAGATGTCCAGGCTGGAGCTGGTCCTTGAAGATCC TAGGGGTCCAGCCCAGCACAGGAGGGCCAGGTGAGAGCCCCCTGTGGTTCTAAGGATGCA ACCAGGGCCGGCGGGTGCCTGCCCTAGAGGGGGTAACTCGGCCCCCTGGGGACCAGTC ACCCCAGGAGGTCCCCAGAGCCCAGCTCGGAGGGCCACAGGTGCCCAGAGTCCCACCTGG GGAAGGCTGCCCCTCCTGCCAGCCCCGAGCCGGGCCCCTGGCGCCCCGCGTCCAGCCGCG ACCCCGGGGAGATATTCACCCCCTGCCCCGTGAATCAGGAGGCCCCGAGCCCATGTTTTCAGTCCTTTTCCTCCCATCCCAGCCCCCCAGGAGAAGAGGTGCTGAACTGGGTCCCCTGG AGGCTCCTGAGCCCCAGAACAGTGCCCTCTGAGCAGACGGGCACTCTCAGACCAGCTCAC GCTGGACAAGTCAGCTCCTGCCTGCCGCCTGATGGGCCCTTGGGAGAAGCAGACATGGTG AGGAAAAGGCCCCTGTGCCCTTCACCCTAATTCCCCAGCCCCAAGTCCCACTGGGTTGCC AGCTTCAACCTAAGCAAATAATTCSTGCCCTCTAAACAAACGCGCGGGAATCCCACCTGC CCTTCCCCCGCCCGCCCCCCC

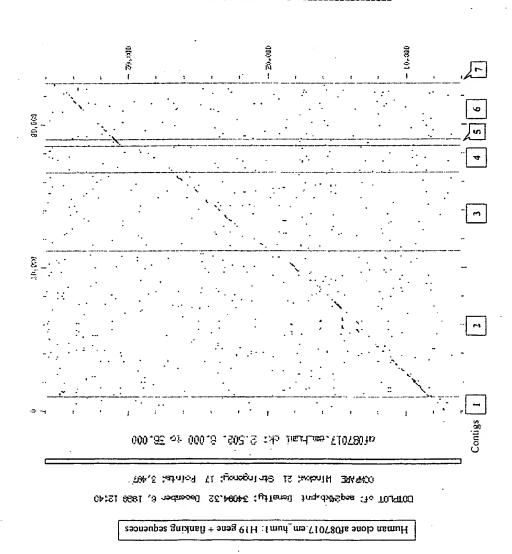
ACCCCTCGCCTTGACCTCCAAAAGCACTTGAGGGGGCTTTCTCCAGACACCCTCCAACCC GAGAGGAGTTGGCGTGGACAGCAGGGGTCAGGCCCCTTTGCCCCGAGGGCAGGGCTGGTG CCACCTGGGTCAGGCGGCAGGCCCTGGAAAAGCACCGGAAATGAGCACACCTGGGTCTCT AGAAGGTTCTTCCAGACCTCTGGGGGCTGAGTCATTTCAACACTCCTGGGCCGGGCAGGG CTTCTTCTTGGCCCCGAGGGACAAGGTCCCCTTCGTCCGGGGGGTACGGCCCCTGGACCC CTGTCCCCCGCACCCCACCCTCCGCCTGGTGAGGUCCGCGGCCAGCTCTGGACACAGATC CCTCAGAGCCCCTTCTCCCTCCTCCTCCTCGTCTTCCCAAGATGCCCCGGCCTCCAGG TGGGGCAGCCAGGCGGCAGAATGTGGTCCAGGCCTCTCGGCCCCACCCCACACCCCCCTGC TCTGCCCTGACAGCCTCCAAGACGCAGGCACGTCGCTGCGTTCTGCGTCCTCTCA TGGCACAAAACGGTGCCCGCCTAGCTTCCCCCAGAGAAGGGAGATCGTGCTCCCCGGACG GACCCTGCTCTGCCTGTCCTCCCGCCCGGCCTTCAGGGCCTCTCCCCAAGGGTGGCCGCG AGGAGGCCCTCGCCTCCGGCCACGGGGGCTCCATCCTCCCGAGCCCGACAGGCCTCCGCC TGGTGGTCCGACCTCTTCCCCAAGGCCCCGCCCATCCTCCTCGCGCTCCCCCAAACCCTG CCTCTTTCCCCAGCGCCCTTGTCCCCACGGAAGACCCTCCACCCGTGCCATTACACGCTC CCGTCTCCCACGGCAGCAGAGGGTCAGCAGCTCAGGGGTCCTGGGGCCGTGGAGATGGCC TGCCCGGGGGTCTCGCTGACCGCCTCCTACGGAAGCTGTGCCGGGGGGTGGGGGGTGTCTC TGCCCGAACGGCTGGAGGACGACCCACATCCCAGGGCAGCCGGAACCTGCGTCCTGGTCT GAGACGGAGAGGCTGGGTGCAGGTGGCTGAGGGCCTGCACACAGCTTGGCCTGGGGTCC CCTAGGTGACAACACTGGCTGAACACTCATTGCTGCTCCCCTTCCAGGGTGACCCTGGGG TCCCCGTGTGGCCCTCAGGGCACACGGGGGCCCCACCAGGCCTCACAGAACCCCAGTGGG ACTGCACCCAGGGCCCACAGAACTGCGGGGGCACTGGGGGTCCAGAAACAACCCCACAAC

CAGGCCAAGGTGGCCAAGGCCTTACTCGAGCGGGGCTGCCCGTCCCAAGAGACTCTGGCC AGTCGTCCGGATCCAGCTTCCCGGGGCCGGGCCGCCCGCTGGGCTCCAGGCGGTTCTGGG GGGCCCTCCCCGGGGGTTCGCCCTCCGCTCTCAGCAGCAGGAAGAGGAGCGCGGCCAGC GGATGGGGAGAAGAGGGCCCCTGGCCATCTTGCTCCCCCTGGGACTTGAGGAGGGTCTC GGGCCGGCAGGCGGGACCGGGAGCCACAGAGACCCTGGAGGAGGCAGCATGGCGGGGAG GGAGGCGTGCCGCTGACCGCCTGGCCGGGAGGTTTGCTGCGTGTGGGGTTTGCAGAAAGT CCTCGGGCACTGCTGACCCATCTCCCGTTTCCAGGGCACCAGAGCCACCTAATCTGCCGG CTCTGTGCCCAGGGACAGGCTTGCCTGATCTCTCAAGGCCGGGCGCTCCGCCTTCCCTGG GAGAGGCTTAAACATCCAGCCCAGCCAGCATCTCGGGCAGCTTCCTGGCTCCCCCCGCT CGTGCCTCCTCTGAGACCCTGGTCGGCACACCTTTCCCTTGAGAGGAGGAGGAGGAGGAA AGCGGATGGAACCAGTGACCCTUCAGCCCCTGAGGGCACCTTCCCACGTGCCCCGCCCG CCCCGCGTCCTCCGCCCCCAGTTCTCACGGCCCCAGTCCTGA'IGGAGGGAGGGCGACCTC CGGGCTCCCTGGCTCCCGCGGGCTCCGGAAGACAGGGCCGCTCGGCTGCGGCTGCAGGGA GGGGCCCUAGACGCAGGAGAGCAGCCCGGAGGCAAACCCCGGGGGTCTTCCAGAAGGAGG CCTGGCAGGGGGGGGGGGGCCACCACTGCTGTCCCTCTCGTGCCACAGTGGAGGGTGT GGGTGGCAGTGCCGGGGTGGGAAGTGCAGAAAGACCCTGGACCGTGGGGCTGGGCCGCC ACGGGGGGCGGGTCTGTCAGGGACCCTGGGGGAGGGAGGCGAAGGGCTGGCCCAGAGG CCGGATCACTTCCAGATTTGCTGTGGGACCAAGGGCCGGACCTCGGGGTGACTTCTTTTG TGTGCTGGCCACAGGGGGCCCCGGCGAGGTCACACGGAAGGGGGCTTCGGACCTGGCCT AACAAGCCCACTCCCGAGGAAGATGCAAGGGGGAGCCAGACGGAAGGGCCGAAGGGGCCGA TCGGGGGACACCGCGCAGGGCCGGGCAGAGAAGGGAGGCAGAGGGAAGGGACC CAGAGGGCAGAGAGGGCAGAGGGGCCACATGCTTGGAGGGCCAGGGAGGAGCGGGA ACGGCGTCCGGCGCCGAATCAGGCCCGTCAGGCGGAGGGTGCGTGGACCTGCC TGGCCTTCACGAGCACAGTCAGCAGGUTGTCTCTTATACACATUTCAACCATCAT

#### Contig 7 (482 bp)

FIGURE 9

Human clone af087017.em\_huml: H119 gene + flanking sequences



SUBSTITUTE SHEET (RULE 26)

FIGURE 10

#### IDENTIFIED POLYMORPHISMS:

POLYMORPHI	SMS TYROSINE HYDROXYLASE GENE - CONT	IS C3 (	figure 6)
1	GGATCCAGCC (A:T) GCAGCC	1081	bp
2	ACAACCCCC ( -: C) TCCCACAG	1149	bp
3 .	TGCGGAGGGG (A:G) GACCTG	1186	bp
4	AGGT (CAAGGCCAGGT: -) CGAGG	1210	bp
POLYMORPHI	SMS INSULIN-IGF2 - CONTIG C4 (figure	6)	
5	CCC (C:A) CCCC (A:C) CGCCGC	438	рb
6	CCC (C:A) CCCC (A:C) CGCCGC	443	рр
7	CGCCGCAGCA (G:A) GCCG	455	pp
8	GCTTATGG (G:A) GCCGGG	503	bp
9	CACGGC (T:C) TC (G:A) GAGCA	525	bp
10	CACGGC (T:C) TC (G:A) GAGCA	528	bp
11	GTCTGC (A:G) GGCAGGTG	571	bp
12	CAAGCCCGG (G:T) CGGTT	636	рb
13	ACCTC (A:G) AGGCCCCCA	710	bp .
14	GC (C:T) GGGCCCAGCCGC	867	bp
15	ACCAGCTG(C:T)GTTCCC	903	bp .
16	GGC (C:G) CTCTGGGCGCC	1148	bp
17	GGGGG (C:T)GTCCCGGGA	1305	dq

WO 00/36143	48/48		PCT/EP99/10209
FIGURE 10,	CONTD.		
18	GCGGT (C:T) GGGGGAGTT	1320 bp	
19	CGCCC(C:T)GGTCCCGCT	1400 bp	
20	TCCC(G:A)TCTGCCGGCC	1519 bp	
21	GA (T:A) GCCCCATCCCCC	1547 bp	
22	GG (C:T) GGCTGCTGCGGC	1607 bp	
23	TGGCTGC (G:A) GTCTGGG	2222 bp	•
<u>POLYMORPHIS</u>	MES IN CODING REGION - CONTIG C10	(figure 6)	
24	GCGCA (G:T) TGATTGGCA	341 bp	
25	CGCCCCCCC(-:c) (G:C)GG	2247 bp	
26	CGCCCCCCC(-:C) (G:C)GG	2248 bp	
27	GCAGCCGGCTC(C:T)TGG	2257 bp	

29	PIGQTL1:	(AT) 11	112	to	133	bp	Contig	57	
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GTTGTTG (C:T) TCTGGGA

**30** *PIGQTL2*: (GT) <sup>8</sup> gcaccccrgrgcccrgrgcac (GT) <sup>17</sup> 1074 to 1144 bp Contig

31 PIGQTL3: (CA) 19 223 to 260 bp Contig 105

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